REMARKS

This Amendment After Final is submitted in reply to the Office Action mailed on June 16, 2004. In the Office Action, the Examiner withdrew newly submitted claims 31-37 as allegedly being directed to a non-elected invention. Also, in the Office Action, the Examiner rejected claims 1-5 and 13-30. With this Amendment After Final, claims 1-5 and 31-37 are canceled, claims 13-30 are amended, and new claims 38-49 are added. Upon entry of this Amendment After Final, the above-identified application will include claims 13-30 and 38-49.

Though claims 1-5 and 31-37 are canceled via this Amendment After Final, Applicant continues to believe claims 1-5 and 31-37 are allowable, as originally presented in the above-identified application and as these claims presently exist as of the present request to cancel these claims. Likewise, though claims 13-30 are amended via this Amendment After Final, Applicant continues to believe claims 13-30 are allowable, as originally presented in the above-identified application and as these claims presently exist as of the present request to amend these claims. Therefore, Applicant is canceling claims 1-5 and 31-37 and amending claims 13-30 without prejudice to Applicant's right to pursue claims worded like claims 1-5 and 31-37, as originally presented or as worded subsequent to original presentation, in the above-identified application or in a continuation application that is based on the above-identified application.

Furthermore, no claim amendment made herein is related to any statutory patentability requirement unless expressly stated herein. Also, no claim amendment made herein is made for the purpose of limiting (narrowing) the scope of any claim.

Examiner's Designation of the Present Office Action as Final

The Examiner's designation of the present Office Action as final based on the Amendment filed on March 19, 2004 is improper since the Examiner introduced a new ground of rejection in the present Office Action when rejecting claims 1-5 under the first paragraph of 35 U.S.C. §112. According to the Examiner:

> For clarification, as supported by the references provided by Applicant, an allelic variant of a given gene is a naturally occurring molecule which differs in sequence (by insertion, deletion, substitution). In other words, the point the Examiner was attempting to make was that an allelic variant is a product which occurs in nature and is not just any variant sequence of gene. There can sometimes be hundreds of allelic variants for a given gene and sometimes there are none.
>
> At page 16 of the response, Applicant points to a definition of 'allelic variant', and asserts that it is consistent with the terminology used in the disclosure. The Examiner does not disagree with the definition cited by Applicant, but again, must emphasize that 'allelic variant' refers to a naturally-occurring molecule(s) with a precise nucleic acid sequence. Based on the reference cited by Applicant, one of ordinary skill in the art would interpret 'an one or more of alternative forms of a given gene' as referring to alternative forms that occur in an organism, absent evidence to the contrary. This differs from any other variant which have been modified for expression in bacteria. These nucleic acid molecules would be variant molecules, but in no way could they be construed as 'allelic variants' within the art recognized meaning of the term, absent evidence to the contrary.

When the Examiner argued that an allelic variant is only a naturally-occurring molecule and only a product which occurs in nature, the Examiner introduced a new basis for rejecting claims 1-5 under the first paragraph of 35 U.S.C. §112. The fact that the Examiner has previously rejected claims 1-5 under the first paragraph of 35 U.S.C. §112 does not matter. The problem is the Examiner's characterization of an allelic variant was not presented in the prior Office Action, and therefore, Applicant was not given an opportunity to respond to the Examiner's improper characterization of allelic variants prior to the Examiner designating the Office Action as final. In essence, the Examiner's new argument regarding the allegedly "naturally-occurring" nature of allelic variants is akin to the Examiner relying upon a new reference in a prior art rejection. This new basis for rejecting claims 1-5 arose despite the fact that claims 1-5 were not amended in reply to the prior Office Action.

The Examiner introduced a new basis for rejecting claims 1-5 under the first paragraph of 35 U.S.C. §112 that was not necessitated by Applicant's amendment of claims 1-5 nor based on information newly submitted in an information disclosure statement. Consequently, the Examiner's designation of the present Office Action as final is erroneous. Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the finality of the present Office Action.

Examiner's Objection to the Claims

In the Office Action, the Examiner objected to claims 17 and 18 under 37 C.F.R. §1.75(c) as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. According the Examiner:

> The instant claims depend from base claims which have two requirements: 1) the DNA molecule must encode a bovine adipocyte leptin and 2) must hybridize to a specified sequence. The dependent claims 17-18 place size limitations on the DNA of 'at least 20' or at least 50 bases, which is no where near the necessary size of a DNA which will encode a bovine leptin polypeptide, absent evidence to the contrary. Therefore, the claims do not appear to further limit the claims from which they depend.

Despite the Examiner's comments, Applicant respectfully disagrees with the Examiner's characterization of claims 17 and 18 and with the Examiner's objection to claims 17 and 18.

Claims 17 and 18 each depend from independent claim 13. Independent claim 13 reads as follows:

> *13. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin that hybridizes to a nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*

Therefore, claim 13 defines, in part, an isolated DNA molecule encoding bovine leptin that hybridizes to the nucleotide sequence identified as SEQ ID NO:3 under stringent hybridization conditions. Dependent claims 17 and 18 read as follows:

> *17. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is at least about 20 bases.*
>
> *18. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is at least about 50 bases.*

Hence, claim 17 specifies the isolated DNA molecule that encodes for bovine leptin polypeptide possess a length of at least about 20 bases. Similarly, claim 18 specifies the isolated DNA molecule that encodes for bovine leptin polypeptide possess a length of at least about 50 bases.

The Examiner's comments suggest it is improper for claims 17 and 18 to define structural features of the isolated DNA in terms of functional attributes by stating isolated DNA molecules which encode for bovine leptin polypeptide and are at least about 20 or at least about 50 bases in length. Nevertheless, the Examiner's characterization of the "at least about 20 bases" and the "at least about 50 bases" as a "size limitation" is inaccurate for several important reasons.

First, it is noted that definition of isolated DNA molecules having at least about 20 or at least about 50 bases (nucleotides) and which encode bovine leptin polypeptide, as defined in claims 17 and 18, is fully supported by the above-identified application. See for example, [0042] of the above-identified application and column 5, lines 40-56, of U.S. Patent No. 6,297,027, issued to Spurlock, hereinafter referred to as the "Spurlock patent."

Next, the Examiner's characterization is inaccurate since claims 17 and 18 contain the term "at least," which is an open ended term requiring that the isolated DNA molecules have a minimum of about 20 bases and about 50 bases, respectively. Furthermore, a person of ordinary skill in the art would typically know to use the isolated DNA sequences having at least about 20 bases to perform hybridization experiments involving leptin DNA sequences. (See the "Kennes publication of Exhibit A of this Amendment After Final). Additionally, those of ordinary skill in the art know that increasing the sequence length from at least about 20 bases to at least about 50 bases permits attainment of a higher specificity during hybridization experiments, as disclosed in the Spurlock patent and the above-identified application.

Finally, it is noted the term "at least" means "at a minimum" and therefore, the isolated DNA molecules defined in claims 17 and 18 are correctly specified as having at least about 20 bases or at least about 50 bases in order to practice the invention of the above-identified application. The term "at least" is not intended to limit the isolated DNA sequences to only about 20 bases or to only about 50 bases in length, as the Examiner argues. Furthermore, the term "at least" is not intended to suggest that bovine leptin polypeptide can be obtained from a DNA molecule containing only about 20 bases or only about 50 bases in length, as the Examiner alleges. The Examiner's stated basis for rejecting claims 17-18 under 37 C.F.R. §1.75(c) is inaccurate and erroneous. Consequently, Applicant respectfully asks the Examiner to reconsider and withdraw the objection to claims 17-18 under 37 C.F.R. §1.75(c) and that claims 17-18 be allowed.

Claim Rejections Under the Written Description Requirement of the First Paragraph of 35 U.S.C. §112

In the Office Action, the Examiner rejected claims 1-5 and 13-30 under the first paragraph of 35 U.S.C. §112 for allegedly failing to provide an adequate written description. In support of this rejection, the Examiner stated:

> Claims 1-5 are rejected and newly added claims 13-30 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement for the reasons of record in the Office action mailed 22 September 2003. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner's reasons of record "in the Office action mailed 22 September 2003" that are referenced in the recited paragraph from the present Office Action (which refers to claims 1-5, but not to claims 13-30 that were added after the September 22, 2003 mailing date of the prior Office Action) state, in part:

> Claims 1-5 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.
>
> In so far as the instant claims are directed to allelic variants of SEQ ID NO:3, the specification lacks an adequate written description of this subject matter. The recitation of 'allelic variant' is directed to a specific molecule for which the instant specification fails to describe the molecule in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The structure of an 'allelic variant' cannot be predicated on the basis of the nucleotide sequence of SEQ ID NO:3 since there is no disclosure of where the variation occurs in the sequence of SEQ ID NO:3. The claims are directed to a species of nucleic acid, the structure of which cannot be determined or predicted from the disclosed nucleic acid sequence and the specification does not evidence isolation or conception of the structure

> of an 'allelic variant', therefore the specification does not provide an adequate written description of the claimed subject matter, and thus the claimed invention, to the extent that it reads upon an 'allelic variant' was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

As noted above, claims 1-5 have been canceled. Next, Applicant notes the Examiner's only stated basis for rejecting claims 13-30 under the written description requirement of the first paragraph of 35 U.S.C. §112 was the "allelic variant" terminology. Claims 19-21 and 28 are believed allowable, since claims 19-21 and 28 have been amended such that claims 19-21 and 28 no longer recite the "allelic variant" terminology and therefore moot the Examiner's stated basis for rejecting claims 19-21 and 28 under the written description requirement of the first paragraph of 35 U.S.C. §112. However, claims 13-18, 22-27, and 29-30 did not previously and do not presently recite the "allelic variant" terminology of concern to the Examiner. Therefore, since there is not stated basis for rejecting claims 13-18, 22-27, and 29-30 under the written description requirement of the first paragraph of 35 U.S.C. §112, claims 13-18, 22-27, and 29-30 are likewise believed allowable.

Claims 13-30 are believed allowable. Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of claims 13-30 under the written description requirement of the first paragraph of 35 U.S.C. §112 and that claims 13-30 be allowed.

Though claims 1-5 are canceled via this Amendment After Final, Applicant continues to believe claims 1-5 are allowable, as originally presented in the above-identified application and as these claims presently exist as of the present request to cancel these claims. Likewise, though claims 19-21 and 28 are amended via this Amendment After Final, Applicant continues to believe claims 19-21 and 28 are allowable, as originally presented in the above-identified application and as these claims presently exist as of the present request to amend these claims. Therefore, Applicant is canceling claims 1-5 and amending claims 19-21 and 28 without prejudice to Applicant's right to pursue claims worded like claims 1-5, 19-21, and 28, as originally presented or as worded subsequent to original presentation, in the above-identified application or in a continuation application that is based on the above-identified application.

Claim Rejections Under the Enablement Requirement of the First Paragraph of 35 U.S.C. §112

In the Office Action, the Examiner rejected claims 14-15 and 17-20 under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the enablement requirement. In support of this rejection, the Examiner stated:

> The claim(s) contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.
>
> The instant claims are directed to nucleic acid molecules which encode a bovine leptin polypeptide, wherein the nucleic acid hybridizes to at least 20-50 bases of SEQ ID NO:3, or wherein the nucleic acid molecule is at least 20-50 bases long. First, the art does not recognize a nucleic acid as short as 20-50 nucleotides long that encodes a leptin molecule and the instant specification fails to teach a molecule meeting this limitation. Therefore, one of ordinary skill in the art would not find such a length sufficient for encoding a leptin molecule from cattle, absent evidence to the contrary, and the claims are not enabled for such. Next, claims 14-15 indicate that the isolated DNA will hybridize to at least 20 or 50 nucleotides of SEQ ID NO:3, however, the vast majority of the nucleic acid molecules which hybridize (no conditions are provided, so the majority of nucleic acids in existence would hybridize under various conditions) to 20 or 50 bases would not meet the functional requirements of the claims, which are to encode a bovine leptin polypeptide. To suggest that one could then test each molecule for functional activity is not an enabling disclosure since the majority of nucleic acids from the cow would hybridize (DNA is inherently sticky) but would not be expected to encode a leptin molecule. Therefore, the claims are not enabled.

Despite the Examiner's comments, claims 14-15 and 17-20 are enabled by the disclosure in accordance with the first paragraph of 35 U.S.C. §112.

The Examiner's rejection of claims 14-15 and 17-20 under the first paragraph of 35 U.S.C. §112 is concerned with the enablement requirement of the first paragraph of 35 U.S.C. §112. The middle portion of the first paragraph of 35 U.S.C. §112 addresses the enablement requirement:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms, as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same . . . .

The enablement requirement is thus concerned with whether the specification disclosure teaches how to make and use the invention defined in the claims.

If the specification disclosure contains this teaching, the claims must be considered to be enabling under the first paragraph of §112 unless the Examiner explains why the Examiner doubts the truth or accuracy of any enabling statement in the disclosure. Otherwise stated, the Examiner has the initial burden of "setting forth a reasonable factual explanation, based on the record as a whole, as to *why* the Examiner believes the scope of protection provided by the claims is not adequately enabled by the description of the invention that is defined in the claims." In re Wright, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993). Furthermore, under Wright, the Examiner must back up assertions controverting the truth and accuracy of enabling statements with acceptable evidence or reasoning as to why the enabling statement is believed untrue or inaccurate.

Applicant asserts the disclosure of the above-identified application does in fact enable claims 14-15 and 17-20. The above-identified application discloses bovine leptin polypeptide and nucleic acid molecules that encode bovine leptin polypeptide. The application further discloses (1) methods of isolating and identifying genes and oligonucleotides that encode for bovine leptin polypeptide and methods for using the isolated genes and oligonucleotides (DNA sequences) to synthesize bovine leptin polypeptides. See column 6, lines 33-48, Example I, and Example III of the Spurlock patent, and [0010], [0014], [0015], [0048], and [0051] of the above-identified application. The disclosure also includes methods to determine the susceptibility of a cow to fat deposition for example using the genes, oligonucleotides and polypeptides for bovine leptin. See column 3, lines 4-17, and column 9, line 30, to column 10, line 10, of the Spurlock patent and [0017] of the above-identified application.

Claims 14 and 15 each depend from independent claim 13. Independent claim 13 reads as follows:

> *13. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin that hybridizes to a nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*

Therefore, claim 13 defines, in part, an isolated DNA molecule encoding bovine leptin that hybridizes to the nucleotide sequence identified as SEQ ID NO:3 under stringent hybridization conditions. Claims 14 and 15 each depend from independent claim 13 and read as follows:

> *14. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule hybridizes to at least about 20 bases of the nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*
>
> *15. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule hybridizes to at least about 50 bases of the nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*

Thus, dependent claims 14 and 15 specify that the isolated DNA molecules of claim 13 hybridize to at least about 20 bases and to at least about 50 bases of the nucleotide sequence identified as SEQ ID NO:3, respectively, under stringent hybridization conditions.

Next, claims 17 and 18 each depend from independent claim 13 and read as follows:

> *17. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is at least about 20 bases.*
>
> *18. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is at least about 50 bases.*

Hence, claims 17 and 18 specify the isolated DNA molecules that encode for bovine leptin polypeptide possess a length of at least about 20 bases and a length of at least about 50 bases, respectively.

Next, claims 19 and 20 each dep end from independent claim 13 and read as follows:

> *19. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is capable of hybridizing to at least about 20 bases of the nucleotide sequence of SEQ ID NO:3 under stringent hybridization conditions.*
>
> *20. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule is capable of hybridizing to at least about 50 bases of the nucleotide sequence of SEQ ID NO:3 under stringent hybridization conditions.*

Thus, claims 19 and 20 specify that the isolated DNA molecules hybridize to at least about 20 bases and to at least about 50 bases of the nucleotide sequence identified as SEQ ID NO:3, respectively, under stringent hybridization conditions.

In support of the rejection, the Examiner alleged, without any evidentiary support, "the art does not recognize a nucleic acid as short as 20-50 nucleotides long that encodes a leptin molecule." This statement of the Examiner, besides lacking evidentiary support, is erroneous. Indeed, the scientific literature recognizes a nucleic acid molecule as short as 17 nucleotides long that is based on the a leptin gene that encodes a leptin molecule used to amplify portions of other leptin genes. More specifically, as disclosed in the second paragraph of the article by Y.M. Kennes, B.D. Murphy, F. Pothier and M.-F Palin, entitled Characterization of Swine *Leptin (Lep)* Polymorphisms and Their Association with Production Traits (2001), primer sequences (nucleic acid molecules) 17 to 21 nucleotides long were used to amplify portions of the leptin genes present in the animals. (Attached as Exhibit A of this Amendment After Final and referred to herein as the "Kennes publication"). Thus, despite the Examiner's allegation to the contrary, the scientific literature does indeed recognize nucleic acid molecules having at least about 20 bases of a nucleotides sequence derived from a leptin gene that encodes a leptin molecule.

Next, contrary to the Examiner's comments ("the instant specification fails to teach a molecule meeting this limitation"), the specification of the above-identified application does indeed teach a nucleic acid molecule possessing at least about 20 nucleotides, as defined in claims 14, 17, and 19; for example, the specification discloses at [0011] of the published version of the present application and at column 2, lines 26-31, of the Spurlock patent:

> The DNA molecule is preferably a single or double stranded DNA molecule having a nucleotide sequence consisting essentially of at least about 20 nucleotides of the nucleotide sequence depicted in FIGS. 1 and 2 (SEQ ID NO:3) or a sequence complementary to at least part of the nucleotide sequence depicted in FIGS. 1 and 2 (SEQ ID NO:3), or an allelic variant thereof, substantially free of other bovine DNA sequences.

The Examiner's comments with regard to claims 14, 17, and 19 and with regard to claims 15, 18, and 20 are further contradicted by the specification disclosure starting at [0048] of the published version of the present application and at column 5, lines 50-56, of the Spurlock patent:

> The DNA sequence should preferably have about 20 or more nucleotides to allow hybridization to another polynucleotide. In order to achieve higher specificity of hybridization, characterized by the absence of hybridization to sequences other than those encoding the polypeptide, or a functional derivative thereof, a length of at least about 50 nucleotides is preferred.

Furthermore, the Spurlock patent (at column 6, lines 26-29) and [0053] of the published version of the above-identified application state:

> Oligonucleotides representing a portion of the bovine adipocyte polypeptide are useful for screening for the presence of genes encoding such proteins and for the cloning of bovine adipocyte polypeptide genes.

Consequently, the specification of the present application clearly and indisputably teaches oligonucleotides (nucleic acid molecules) having a sequence length of at least about 20 bases and at least about 50 bases are useful for the identification and cloning of additional bovine DNA sequences and polypeptides that have homologous sequences to the sequences of the bovine leptin polypeptides disclosed in the present application.

The Examiner's enablement rejection of claims 14-15 and 17-20 is based on the faulty and unsupported premise that "the art does not recognize a nucleic acid as short as 20-50 nucleotides long that encodes a leptin molecule." This premise is wrong, as established above. Furthermore, the present application teaches, as established above, nucleic acid molecules with a sequence length of at least about 50 bases, and even as short as at least about 20 bases, are useful for identifying and cloning additional bovine DNA sequences and polypeptides with sequences homologous to the bovine leptin polypeptides disclosed in the present application.

Consequently, claims 14-15 and 17-20 are clearly enabled by the specification of the above-identified application. The specification of the present application and even the scientific

literature disclose such oligonucleotides on the order of about 20 bases long, or more, and teach use of such molecules in hybridization studies.

Additionally, the Examiner's comments regarding testing isolated DNA molecule having at least about 20 nucleotides for functional bovine leptin activity are erroneous; therefore, these comments of the Examiner do not provide support for the Examiner's rejection of claims 14-15 and 17-20 under the enablement requirement. The Examiner's comments on this point are erroneous since the Examiner suggests the only application for using DNA sequences that encode for bovine leptin activity is generation of functional bovine leptin polypeptide. As noted above and as disclosed in the present application, DNA sequences of at least about 20 nucleotides are useful for isolating and identifying genes and oligonucleotides that encode for bovine leptine polypeptides. Also, as noted above and as disclosed in the present application, DNA sequences of at least about 20 nucleotides are useful for determining the susceptibility of a cow to fat deposition. The comments demonstrate the Examiner's enablement rejection is faulty and without basis and that claims 14-15 and 17-20 are in fact enabled by the specification of the above-identified application.

Applicant has demonstrated that claims 14-15 and 17-20 are enabled by the specification of the above-identified application. The application teaches the generation and use of DNA sequences at least about 50 nucleotides long and even as short as at least about 20 nucleotides long. Applicant has provided factual evidence via the Kennes publication illustrating that DNA sequences with a length of at least about 20 nucleotides are useful in hybridization trials involving leptin genes. Furthermore, the present application enables use of DNA sequences of at least about 20 nucleotides for isolating and identifying genes and oligonucleotides that encode for bovine leptine polypeptides. Also, as noted above, the present application enables use of DNA sequences of at least about 20 nucleotides for determining the susceptibility of a cow to fat deposition.

Since the specification disclosure does contain an enabling disclosure of at least the same breadth as the scope of claims 14-15 and 17-20, the Examiner must consider claims 14-15 and 17-20 to be enabled under the first paragraph of §112 unless the Examiner explains why the Examiner doubts the truth or accuracy of any enabling statement provided in the above-identified

application. The Examiner has the initial burden of "setting forth a reasonable factual explanation, based on the record as a whole, as to *why* the Examiner believes the scope of protection provided by the claims is not adequately enabled by the description of the invention that is defined in the claims." In re Wright. Furthermore, under Wright, the Examiner must back up assertions controverting the truth and accuracy of enabling statements with acceptable evidence or reasoning as to why the enabling statement is believed untrue or inaccurate. The Examiner has not demonstrated that the present invention, as defined in claims 14-15 and 17-20, is broader than the scope of the enabling disclosure. Furthermore, the Examiner has not produced any evidence that controverts the veracity of any specification statement that enables the present invention, as defined in claims 14-15 and 17-20. Therefore, claims 14-15 and 17-20 are believed enabled by the specification of the above-identified application.

Claims 14-15 and 17-20 are believed allowable. Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of claims 14-15 and 17-20 under the enablement requirement of the first paragraph of 35 U.S.C. §112 and that claims 14-15 and 17-20 be allowed.

Claim Rejections Under the Second Paragraph, 35 U.S.C. §112

In the Office Action, the Examiner rejected claims 13-30 under the second paragraph of 35 U.S.C. §112 as allegedly "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." The Examiner's specific remarks in regard to this rejection were:

> Claims 13-30 are directed to nucleic acid molecules (DNA, mRNA) which 'hybridizes' to a particular disclosed nucleic acid sequence, wherein no hybridization conditions are provided. Some of the claims recite that a certain number of bases will hybridize (at least 20, at least 50), or that 'substantially' all of the bases will hybridize, or 'under hybridizing conditions'. However, these claims are indefinite for the failure to indicate what hybridization conditions are to be used or what degree of identity is intended with 'substantially all'. Without knowing what conditions are to be used, the skilled artisan

> would not know if a molecule which may be isolated by using the disclosed nucleic acid molecule will be encompassed by the claims because the metes and bounds of what is claimed is not clear.

Despite the Examiner's allegations, claims 13-30 are believed definite in accordance with the second paragraph of 35 U.S.C. §112.

The Examiner's comments relate to the "under hybridizing conditions" terminology used in some of claims 13-30 and to the "substantially all" terminology used in some of claims 13-30. Claims employing the "substantially all" terminology are addressed first.

Claims 16, 21, 23-24, and 26-29 each employ the "substantially all" terminology of concern to the Examiner. The comments provided below in support of the definiteness of claim 16 that employs the "substantially all" terminology are equally applicable to claims 21, 23-24, and 26-29.

Claim 16 depends from independent claim 13 which reads as follows:

> *13. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin that hybridizes to a nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*

Continuing, claim 16 reads as follows:

> *16. (Currently Amended) The isolated single or double-stranded DNA molecule of claim 13 wherein the isolated DNA molecule hybridizes to substantially all of the bases of the nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.*

Thus, claim 16 recites an isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin that hybridizes to substantially all of the bases of the nucleotide sequence of SEQ ID NO: 3 under stringent hybridization conditions.

The second paragraph of 35 U.S.C. §112 is concerned with whether those skilled in the art will be able to understand with a reasonable degree of accuracy what subject matter is circumscribed by the invention that is defined by a particular claim. If those skilled in the art can reasonably determine whether any particular subject matter either falls within the scope of a particular claim or falls outside the scope of the particular claim, that claim is not indefinite or ambiguous under the second paragraph of 35 U.S.C. §112. Miles Labs v Shandon, 27 USPQ2d 1123, 1123 (Fed Cir. 1993). Furthermore, the mere fact that the "substantially all" terminology of

claim 16 is broader that stating a specific numeric determinant does not render a claim containing this language indefinite. See In re Miller, 441 F.2d 689, 169 USPQ 597 (CCPA 1971).

Applicant notes that applications handled by the Examiner have issued into U.S. patents with claims than employ "substantially all" terminology. For example, claim 1 of U.S. Patent No. 6,756,484 employs the "substantially all" terminology more than five different times. In one instance, claim 1 recites:

> (C) . . . with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace **substantially all** of said authentic and non-authentic IGF-I from said cation exchange matrix . . . .

Emphasis added. Applicant's review of U.S. Patent No. 6,756,484 did not identify any particular numeric meaning or degree of identity for this use of the "substantially all" terminology. Indeed, the Courts do not require that any particular numeric meaning be provided for a claim containing the "substantially all" terminology to be definite. The Examiner asks "what degree of identity is intended" for Applicant's use of the "substantially all" terminology in the claims at issue. However, like numeric meaning, the Courts do not require that any "degree of identity" be provided for a claim containing the "substantially all" terminology to be definite. Rather, the question is whether those skilled in the art will be able to understand with a *reasonable* degree of accuracy what subject matter is circumscribed by the invention that is defined by a particular claim, such as claim 16. Also, the issue is not whether the particular terminology is definite, but rather whether the meaning of the claim containing the terminology at issue is definite.

Some information about the meaning of the "substantially all" terminology is readily evident from the word itself. For example, "substantially all" clearly means something less than "all." Also, it is safe to say the "substantially all" means something more than "half." Beyond this, the "under stringent hybridization conditions" terminology of claim 16 provides further guidance.

Nucleic acid hybridization entails base pairing between two nucleic acid molecules, such as a single-stranded nucleic acid molecule of a defined sequence (probe) and a second nucleic acid molecule (target) with a sequence complementary to the sequence of the probe, to form a hybrid (duplex) molecule. Hybridization with Analysis of DNA Blots. Current Protocols in Molecular Biology, Section

II 2.10.1. (John Wiley & Sons 2000) (attached as Exhibit B of this Amendment After Final); and Nucleic Acid Hybridization, pp 8-1 to 8-4, obtained at http://dir.niehs.nih.gov/dirlep/files/hybridiz.pdf on 11-19-04 (attached as Exhibit C of this Amendment After Final). The stability of the hybrid molecule formed by virtue of the hybridization depends on the extent of base-pairing that occurs between the target and the probe. (Exhibit B and Exhibit C of this Amendment After Final). Therefore, a high degree of base-pairing between the target and the probe results in a more stable hybrid wherein the target and the probe remain associated with each other; conversely, a low degree of base-pairing between target and probe results in a less stable hybrid (duplex) wherein the target and the probe have an increased tendency to dissociate from each other. (Exhibit B and Exhibit C of this Amendment After Final).

Furthermore, those of ordinary skill in the art know a high degree of base-pairing in a hybrid molecule occurs when both target and probe possess sequences that are well-matched (complementary) to each other. (Exhibit B and Exhibit C of this Amendment After Final). In addition, a person of ordinary skill in the art knows little or no mismatch (pairing of non-complementary sequences) occurs between a probe and a target when stringent conditions are used during hybridization. (Exhibit B and Exhibit C of this Amendment After Final). This is because stringent hybridization conditions will allow only well-matched ("perfect or near perfect") hybrid molecules to form where a high degree of base-pairing is present between the DNA probe and the target DNA molecule. (Exhibit B of this Amendment After Final, also see Exhibit C of this Amendment After Final).

Claims 16, 21, 23-24, and 26-29 that each employ the "substantially all" terminology of concern to the Examiner also recite "stringent hybridization conditions." As noted above, one of ordinary skill in the art knows that under stringent hybridization conditions, a high degree of base-paring of the DNA probe to the target DNA molecule will result and only well-matched ("perfect or near perfect") hybrid molecules based on the highly or fully complementary sequences of the DNA probe and the target DNA molecule will form. Consequently, one of ordinary skill in the art would understand the "substantially all" term of claims 16, 21, 23, 24, and 26-29 characterizes the high ("perfect or near perfect") degree to which the DNA probe base-pairs to the target DNA

molecule. The term "substantially all" merely describes the high degree to which two DNA molecules are capable of hybridizing to each other under stringent hybridization conditions.

The foregoing comments demonstrate one of ordinary skill in the art of microbiology would be able to understand, with a *reasonable* degree of accuracy, what subject matter is circumscribed by the invention defined by claims 16, 21, 23-24, and 26-29 which employ the "substantially all" terminology. First, the meaning of the term "substantially all" clearly means something less than "all," yet more than "half." Beyond this, the "under stringent hybridization conditions" terminology of claims 16, 21, 23-24, and 26-29 provide ample further guidance. Specifically, one of ordinary skill in the art would understand the "substantially all" term of claims 16, 21, 23, 24, and 26-29 characterizes the high ("perfect or near perfect") degree to which the DNA probe base-pairs to the target DNA molecule.

As noted, some of the Examiner's comments recited above relate to the "under hybridizing conditions" terminology used in some of claims 13-30. Claims 13-16 and 19-30 each recite hybridization or the capability of undergoing hybridization. Claims 17 and 18 depend from claim 13. The most pertinent comments of the Examiner in relation to the specified hybridization were:

> Claims 13-30 are directed to nucleic acid molecules (DNA, mRNA) which 'hybridizes' to a particular disclosed nucleic acid sequence, wherein no hybridization conditions are provided. Some of the claims recite . . . 'under hybridizing conditions.' However, these claims are indefinite for the failure to indicate what hybridization conditions are to be used . . . .

Claims 13-16 and 19-30 now recite the hybridization occurs under stringent hybridization conditions. This is believed to adequately address the Examiner's rejection of claims 13-30 under the second paragraph of 35 U.S.C. §112 on the hybridization condition basis.

The above-identified application provides ample support for the 'stringent hybridization conditions" terminology recited in claims 13-16 and 19-30. A Declaration under 37 C.F.R. §1.132 by Dr. Michael Spurlock is attached as Exhibit D of this Amendment After Final. Exhibits A-W that are referenced in Dr. Spurlock's §1.132 Declaration are also attached as Exhibits A-W of the §1.132 Declaration. Dr. Spurlock is the sole inventor of the invention described and

claimed in the above-identified application and is also the sole inventor of the invention defined and claimed in U.S. Serial Application No. 08/688,908, now U.S. Patent No. 6,297,027. ¶ 11 of attached §1.132 Declaration.

The invention of the above-identified application is, in one aspect, directed to nucleic acid molecules (and functional variants thereof), such as (1) single or double-stranded DNA (and cDNA and genomic DNA) and (2) RNA (and mRNA), that encode bovine adipocyte polypeptide leptin. ¶ 20 of attached §1.132 Declaration. According to the present invention, the nucleic acid molecules (and functional variants thereof) encoding for bovine leptin polypeptide mentioned in ¶ 20 of the attached §1.132 Declaration hybridize and are capable of hybridizing to nucleotide sequences (and portions thereof), such as various nucleotide sequences (and portions thereof) disclosed in the above-identified application. ¶ 21 of attached §1.132 Declaration.

In another aspect, the invention of the above-identified application is directed to methods of hybridizing the nucleic acid molecules encoding for bovine leptin polypeptide (and functional variants thereof) to nucleotide sequences (and portions thereof), such as various nucleotide sequences (and portions thereof) disclosed in the above-identified application. ¶ 22 of attached §1.132 Declaration. Examples II and III of the above-identified application provide particular guidance about hybridization conditions that may be employed when practicing the hybridization methods of the present invention. ¶¶ 23 and 45 of attached §1.132 Declaration. This guidance of Examples II and III illustrates that stringent hybridization conditions are suitable for practicing the hybridization methods of the present invention. ¶¶ 23 and 45 of attached §1.132 Declaration.

The formation of a duplex by nucleic acid hybridization (base pairing between two nucleic acid molecules) is directly related to the degree of stringency of the hybridization conditions employed. ¶ 12 of attached §1.132 Declaration. Persons of ordinary skill in the art of molecular biology know high temperature is an example of a stringent hybridization condition. ¶ 13 of attached §1.132 Declaration. Persons of ordinary skill in the art of molecular biology know another example of a stringent hybridization condition is the chemical composition of the hybridization solution, such as hybridization solution with relatively low ionic strength (i.e. a relatively low salt concentration).

¶ 14 of attached §1.132 Declaration. Thus, persons of ordinary skill in the art of molecular biology know the stringency of hybridization conditions may be adjusted by varying the temperature at which hybridization is performed, the chemical composition of the hybridization solution used during hybridization experiments, or both the hybridization temperature and the chemical composition of hybridization solution. ¶ 15 of attached §1.132 Declaration.

Persons of ordinary skill in the art of molecular biology know time of exposure to hybridization solution at a particular temperature may also be manipulated to attain stringent hybridization conditions; for example, hybridization overnight for a time ranging from twelve to sixteen hours is generally sufficient for most probes and blots to complete base pairing between a nucleic acid sequence and a DNA or mRNA sequence. ¶ 16 of attached §1.132 Declaration. Persons of ordinary skill in the art of molecular biology also know another technique for improving specificity of base pairing entails incorporation of non-specific DNA in the hybridization solution. ¶ 17 of attached §1.132 Declaration. Persons of ordinary skill in the art of molecular biology know one example of non-specific DNA that may be used to block hybridization of non-specific DNA with the probe nucleic acid is salmon sperm. ¶ 18 of attached §1.132 Declaration.

Furthermore, beyond controlling the hybridization stringency directly, by varying the temperature at which hybridization is performed and/or the chemical composition of the hybridization solution used during hybridization experiments (including use of blocking substances as described above), those of ordinary skill in the art of molecular biology know hybridization stringency may also be controlled by post-hybridization washing conditions:

> You can also control for stringency of hybridization at the next step, which is washing the unbound probe away from the blot. If you wash at high temperature or low salt concentration, you will remove any hybrids that are not perfectly matched.

¶ 19 of attached §1.132 Declaration (citing Exhibit H of attached §1.132 Declaration. Thus, persons of ordinary skill in the art of molecular biology know the stringency of hybridization conditions may be adjusted by varying the temperature at which hybridization is performed, the chemical composition of the hybridization solution used during hybridization, the time of exposure to the hybridization solution

at a particular temperature during hybridization, incorporation of non-specific DNA (such as salmon sperm) in the hybridization solution, and post-hybridization washing conditions, such as the temperature and/or salt concentration of the post-hybridization wash solution.

We first consider the hybridization conditions taught and disclosed by Example II of the above-identified application. Example II discloses a hybridization technique using bovine leptin cDNA as a probe to detect full length bovine leptin mRNA. ¶¶ 24-25 of attached §1.132 Declaration. According to Example II, bovine leptin cDNA was hybridized against bovine leptin mRNA at 55°C for twenty hours. ¶¶ 24 and 26 of attached §1.132 Declaration. Use of temperatures at and below 55°C (specifically, 50°C in numbered para. 4 on the first page of Exhibit I of §1.132 Declaration, 55°C in numbered para. 4 on the second page of Exhibit I of §1.132 Declaration, and 50°C in numbered para. 4 on the third page of Exhibit I of §1.132 Declaration) is disclosed as adequate to produce stringent conditions using a non-formamide hybridization solution. ¶ 27 of attached §1.132 Declaration (citing Exhibit J of §1.132 Declaration). The evidence provided above regarding use of a hybridization temperature of 55°C during the hybridization trial of Example II of the present application illustrates, to those of ordinary skill in the art of molecular biology, use of stringent hybridization conditions during the hybridization trial of Example II. ¶ 28 of attached §1.132 Declaration. This statement is probative as to stringency even if the salt concentration in the hybridization solution of Example II is not taken into account. See ¶ 15 of attached §1.132 Declaration.

Next, we consider the hybridization time of twenty hours employed in Example II. ¶¶ 24 and 26 of attached §1.132 Declaration. Hybridization overnight for a time ranging from twelve to sixteen hours is generally sufficient for most probes and blots to complete base pairing between a nucleic acid sequence and a DNA or mRNA sequence. ¶ 30 of attached §1.132 Declaration (citing page 3 of Exhibit D of §1.132 Declaration). Others in the molecular biology industry also indicate hybridization overnight (specifically, numbered para. 4 on the first page of Exhibit I, numbered para. 4 on the second page of Exhibit I, and numbered para. 4 on the third page of Exhibit I) is adequate to produce stringent conditions using a non-formamide hybridization solution. ¶ 31 of attached §1.132 Declaration (citing Exhibit I of §1.132 Declaration). The evidence provided above regarding hybridization for twenty hours, which

clearly qualifies as overnight, during the hybridization trial of Example II of the present application, illustrates to those of ordinary skill in the art of molecular biology, stringent hybridization conditions were employed during the hybridization trial of Example II. See ¶ 32 of attached §1.132 Declaration.

Next, we consider the salt concentration employed in the hybridization solution of Example II.. Since the hybridization solution contained 0.9 M NaCl and 0.09 M sodium citrate, one of ordinary skill in the art of molecular biology would understand the hybridization solution contained 0.99 M sodium ion. ¶ 33 of attached §1.132 Declaration (citing ¶ 24 of §1.132 Declaration). WO 02/036829A2 states that salt concentrations of 0.99 M sodium ion are considered to be low salt concentration generally sufficient to establish stringent conditions:

> Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7. 0 to 8.3 . . . .

¶ 34 of attached §1.132 Declaration (citing page 12, lines 2-4, of Exhibit K of §1.132 Declaration). The evidence provided above regarding use of a hybridization solution with a salt concentration of 0.99 M sodium ion during the hybridization trial of Example II of the present application illustrates, to those of ordinary skill in the art of molecular biology, that stringent hybridization conditions were employed during the hybridization trial of Example II. ¶ 35 of attached §1.132 Declaration. This statement is probative as to stringency even if the hybridization temperature of Example II is not taken into account. ¶ 35 of attached §1.132 Declaration (citing ¶ 15 of §1.132 Declaration).

Next, we consider the salmon sperm concentration of 100 μg/ml employed in the hybridization solution of Example II. ¶ 36 of attached §1.132 Declaration (citing ¶ 24 of §1.132 Declaration). Persons of ordinary skill in the art of molecular biology also know another technique for improving specificity of base pairing entails incorporation of non-specific DNA in the hybridization solution. ¶ 37 of attached §1.132 Declaration (citing ¶ 17 and Exhibit E of §1.132 Declaration). The non-specific DNA is said to prevent the probe from hybridizing nonspecifically - that is with nucleic acid that is not complementary (specific) to the probe nucleic acid. ¶ 37 of attached §1.132 Declaration (citing ¶ 17 and Exhibit F of §1.132 Declaration).

As an example, salmon sperm at a concentration of 100 μg/ml is incorporated by those skilled in the art of molecular biology to complement and support high stringency hybridization by limiting non-specific nucleic acid binding during hybridization. ¶ 38 of attached §1.132 Declaration (citing Exhibit L of §1.132 Declaration). The evidence provided above regarding use of a hybridization solution with a salmon sperm concentration of 100 μg/ml during the hybridization trial of Example II of the present application illustrates, to those of ordinary skill in the art of molecular biology, use of conditions that inhibit non-specific probe hybridization and therefore further support attainment of results consistent with use of stringent conditions during the hybridization trial of Example II. ¶ 39 of attached §1.132 Declaration.

We next consider the post-hybridization washing conditions of Example II that entailed washing "to a final stringency of 0.1xSSC (0.015 M NaCl, 0.0015 M sodium citrate), 0.1% SDS at 60°C. ¶ 40 of attached §1.132 Declaration (citing ¶ 24 of §1.132 Declaration). Beyond controlling the hybridization stringency directly, by varying the temperature at which hybridization is performed and/or the chemical composition of the hybridization solution used during hybridization experiments, those of ordinary skill in the art of molecular biology know hybridization stringency may also be controlled by post-hybridization washing conditions, such as the temperature and/or the salt concentration of the wash solution. ¶ 41 of attached §1.132 Declaration (citing ¶ 19 of §1.132 Declaration). For example:

> You can also control for stringency of hybridization at the next step, which is washing the unbound probe away from the blot. If you wash at high temperature or low salt concentration, you will remove any hybrids that are not perfectly matched.

¶ 41 of attached §1.132 Declaration (citing Exhibit H of §1.132 Declaration).

In this regard, those of ordinary skill in the art recognize that high stringency washing may be accomplished using the hybridization temperature in combination with a final washing solution containing 0.2X SSC and 0.1% SDS, as was employed in Example II:

> Following hybridisation, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridisation temperature in 0.2-0.1 times SSC, 0.1% SDS.

¶ 42 of attached §1.132 Declaration (citing ¶ 0188 of Exhibit M of §1.132 Declaration). The foregoing evidence demonstrates those of ordinary skill in the art of molecular biology recognize that high stringency washing may be accomplished using a final washing solution containing 0.2X SSC and 0.1% SDS, as was employed in Example II, and also demonstrates this stringent washing solution may be employed at the hybridization temperature to further enhance the washing stringency. ¶ 43 of attached §1.132 Declaration. Thus, the use of high stringency washing, as was employed in Example II of the above-identified application, illustrates, to those of ordinary skill in the art of molecular biology, use of conditions that further support attainment of results consistent with use of stringent conditions during the hybridization trial of Example II.

Example II of the above-identified application provides particular guidance about hybridization conditions that may be employed when practicing the hybridization methods of the present invention. ¶ 23 of attached §1.132 Declaration. This guidance of Example II illustrates that stringent hybridization conditions are suitable for practicing the hybridization methods of the present invention. ¶ 23 of attached §1.132 Declaration. Furthermore, this guidance of Example II teaches and discloses use of stringent hybridization conditions for practicing the hybridization methods of the present invention.

For instance, Example II discloses use of a hybridization temperature of 55 °C that those of ordinary skill in the art of molecular biology would recognize as a stringent hybridization condition. Also, Example II employs a hybridization solution with a salt concentration of 0.99 M sodium ion that those of ordinary skill in the art of molecular biology would recognize as a stringent hybridization condition. Also, those of ordinary skill in the art of molecular biology would recognize the Example II hybridization for twenty hours as being consistent with stringent hybridization conditions. Continuing, those of ordinary skill in the art of molecular biology would recognize the use in Example II of a hybridization solution with a salmon sperm concentration of 100 µg/ml as being consistent with use of stringent hybridization conditions. Finally, those of ordinary skill in the art of molecular biology would recognize the use in Example II of stringent washing conditions (final washing solution at hybridization temperature and containing 0.2X SSC and 0.1% SDS), as use of conditions that may be part of an overall

stringent hybridization protocol that support attainment of results consistent with use of stringent conditions during the Example II hybridization trial.

The guidance of Example II regarding hybridization conditions illustrates that stringent hybridization conditions are suitable for practicing the hybridization methods of the present invention. Indeed, the evidence provided herein illustrates one of ordinary skill in the art of molecular biology would recognize that the hybridization conditions employed in Example II of the above-identified application constitute stringent hybridization conditions that would typically enable a probe, such as the bovine leptin cDNA molecule employed in Example II, to hybridize to a perfect or near perfect nucleic acid molecule complement (bovine leptin mRNA) of the probe. ¶ 44 of attached §1.132 Declaration (citing ¶¶ 23-43 of the §1.132 Declaration, as supplemented by Exhibits B-D of the §1.132 Declaration and by the Exhibits referenced in ¶¶ 23-43 of the §1.132 Declaration).

Next, we consider the hybridization conditions taught and disclosed by Example III of the above-identified application. Example III discloses a hybridization trial that used bovine leptin cDNA as a probe to screen a bovine genomic DNA library and detect bovine leptin DNA. ¶ 46 of attached §1.132 Declaration.

According to Example III, the bovine leptin cDNA was hybridized against the bovine leptin DNA for twenty-one hours. ¶¶ 45 and 47 of attached §1.132 Declaration. Hybridization overnight is generally recognized by those of ordinary skill in the art of molecular biology as being sufficient for most probes and blots to complete base pairing between a nucleic acid sequence and a DNA sequence. ¶ 48 of attached §1.132 Declaration (citing page 3 of Exhibit D of §1.132 Declaration). The evidence provided above regarding hybridization for twenty-one hours, which clearly qualifies as at least overnight, during the hybridization trial of Example III of the present application illustrates, to those of ordinary skill in the art of molecular biology, stringent hybridization conditions were employed during the hybridization trial of Example III. (See ¶ 49 of attached §1.132 Declaration).

Next, we consider the salt concentration employed in the hybridization solution of Example III. Specifically, since the hybridization solution contained 0.8 M NaCl and 0.02 M pipes buffer (and assuming the pipes buffer employed NaOH), one of ordinary skill in the art of molecular biology

would understand the hybridization solution contained about 0.82 M sodium ion. See ¶ 50 above (citing ¶ 45). Those of ordinary skill in the art of microbiology know salt concentrations of about 0.82 M sodium ion are considered to be low salt concentrations generally sufficient to establish stringent conditions:

> Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7. 0 to 8.3 . . . .

Page 12, lines 2-4 of Exhibit K.

The evidence provided above regarding use of a hybridization solution with a salt concentration of about 0.82 M sodium ion during the hybridization trial of Example III of the present application illustrates, to those of ordinary skill in the art of molecular biology, that stringent hybridization conditions were employed during the hybridization trial of Example II. ¶ 52 of attached §1.132 Declaration. This statement is probative as to stringency even if the hybridization temperature of Example II is not taken into account. ¶ 52 of attached §1.132 Declaration (citing ¶ 15 of §1.132 Declaration).

We first consider the hybridization conditions taught and disclosed by Example III of the above-identified application. Example III discloses a hybridization technique using bovine leptin cDNA as a probe to screen a bovine genomic DNA library and detect bovine leptin DNA. ¶¶ 45-46 of attached §1.132 Declaration. According to Example III, bovine leptin cDNA was hybridized against the bovine leptin DNA for twenty-one hours. ¶¶ 45 and 47 of attached §1.132 Declaration. Hybridization overnight for a time ranging from twelve to sixteen hours is generally sufficient for most probes and blots to complete base pairing between a nucleic acid sequence and a DNA or mRNA sequence. ¶ 48 of attached §1.132 Declaration (citing page 3 of Exhibit D of §1.132 Declaration). The evidence noted above regarding hybridization for twenty-one hours, which clearly qualifies as overnight, during the hybridization trial of Example III of the present application, illustrates to those of ordinary skill in the art of molecular biology, stringent hybridization conditions were employed during the hybridization trial of Example III. See ¶ 49 of attached §1.132 Declaration.

Next, we consider the salt concentration employed in the hybridization solution of Example III. Since the hybridization solution contained 0.8 M NaCl and 0.02 M pipes buffer (and assuming the pipes buffer employed NaOH), one of ordinary skill in the art of molecular biology would

understand the hybridization solution contained about 0.82 M sodium ion. ¶ 50 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). WO 02/036829A2 demonstrates that salt concentrations of about 0.82 M sodium ion are considered to be low salt concentration generally sufficient to establish stringent conditions:

> Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7. 0 to 8.3 . . . .

¶ 51 of attached §1.132 Declaration (citing page 12, lines 2-4, of Exhibit K of §1.132 Declaration). The evidence provided above regarding use of a hybridization solution with a salt concentration of about 0.82 M sodium ion during the hybridization trial of Example III of the present application illustrates, to those of ordinary skill in the art of molecular biology, that stringent hybridization conditions were employed during the hybridization trial of Example III. ¶ 52 of attached §1.132 Declaration. This statement is probative as to stringency even if the hybridization temperature of Example III is not taken into account. ¶ 52 of attached §1.132 Declaration (citing ¶ 15 of §1.132 Declaration).

Next, we consider the 50% formamide concentration employed in the hybridization solution of Example III. ¶ 53 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). Beyond maintaining hybridization solution with relatively low ionic strength (i.e. a relatively low salt concentration) (see ¶¶ 15 and 50-52 of §1.132 Declaration), persons of ordinary skill in the art of molecular biology know another approach to attaining stringent hybridization conditions entails incorporation of formamide in the hybridization solution. ¶ 54 of attached §1.132 Declaration (citing Slide 21 of Exhibit N of §1.132 Declaration). Persons of ordinary skill in the art of molecular biology know addition of formamide at a particular annealing temperature (T_M) enhances hybridization stringency by destabilizing the helical form of the nucleotides being hybridized: "*Formamide* is a helix destabiliser . . . ." ¶ 55 of attached §1.132 Declaration (citing Exhibit O of §1.132 Declaration). By virtue of this helix destabilization, the formamide "'opens' nucleic acid." ¶ 55 of attached §1.132 Declaration (citing slide 19 of Exhibit N of §1.132 Declaration).

Use of formamide at a concentration of 50% in the hybridization solution is generally sufficient to establish stringent conditions:

> After transfer, blots were hybridized at high stringency [50% formamide, 5X sodium chloride-sodium phosphate-EDTA (SSPE) (1X SSPE is 0.15 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA), 5X Denhardt's solution, 0.2% SDS, and 10 μg/ml herring sperm DNA at 42°C] with PEPT1, PEPT2, SGLT1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragments labeled with [α-32P]dCTP as probes.

and:

> Membranes were hybridized either under conditions of moderate stringency [hybridization in 35% formamide, 0.1% Na-pyrophosphate, 50 mM Tris·Cl (pH 7.5), 5× SSC, 1% SDS, 5× Denhardt's, 100 μg/ml calf thymus DNA at 42°C, washing in 1× SSC/1% SDS at 60°C] or high stringency (hybridization in the same buffer except that the formamide concentration was 50% and at the same temperature . . . .

¶ 56 of attached §1.132 Declaration (citing page G58 of Exhibit P and page 11779 of Exhibit Q of §1.132 Declaration). Use of a hybridization solution with a 50% concentration of formamide during the hybridization trial of Example III of the present application thus illustrates, to those of ordinary skill in the art of molecular biology, use of stringent hybridization conditions during the hybridization trial of Example III. ¶ 57 of attached §1.132 Declaration (citing ¶¶ 54-56 of §1.132 Declaration). Furthermore, addition of formamide allows the hybridization temperature to be decreased while maintaining the stringent hybridization conditions that would exist without changing the hybridization temperature and in the absence of the formamide:

> *Formamide* . . . enables one to decrease [the] annealing temperature: the presence of **formamide** decreases the Tm as shown:
>
> **TFm = Tm - 0.61(%formamide, w/v)**

¶ 58 of attached §1.132 Declaration (citing "Hybridization Stringency" section of Exhibit O of §1.132 Declaration).

A pre-hybridization temperature of about 42°C is employed in Example III. ¶ 59 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). As evidenced below, those of ordinary skill in the art of molecular biology would therefore understand the hybridization temperature employed in Example III was also about 42°C. ¶ 59 of attached §1.132 Declaration.

In a particular hybridization run, those of ordinary skill in the art of microbiology know the hybridization temperature is typically about the same as the prehybridization temperature that is employed:

> This step should always be carried out at the temperature of the hybridization.

¶ 60 of attached §1.132 Declaration (citing "Prehybridization" section of Exhibit R of §1.132 Declaration). This knowledge about the hybridization temperature typically being about the same as the prehybridization temperature is further exemplified by various sources. ¶ 61 of attached §1.132 Declaration. For example, compare numbered paragraphs 1 and 4 on the first page of Exhibit I, numbered paragraphs 1 and 4 on the second page of Exhibit I, and numbered paragraphs 1 and 4 on the third page of Exhibit I that each show the hybridization temperature and the prehybridization temperature were the same in any particular hybridization trial. ¶ 61 of attached §1.132 Declaration (citing Exhibit I of §1.132 Declaration). As another example, see:

> The baked membranes were prehybridized using 25 mM potassium phosphate, 750 mM NaCl, 75 mM sodium citrate, 5XDenhardt's solution, 50 µg/ml denatured salmon sperm DNA and 50% formamide. After incubation for 14–16 h at 42°C, the membranes were hybridized with 32P-labeled probes in the prehybridization buffer plus 10% dextran sulfate. After hybridization for 14–16 h at 42°C . . . .

¶ 61 of attached §1.132 Declaration (citing page 351 of Exhibit S of §1.132 Declaration).

A pre-hybridization temperature of about 42°C is employed in Example III. ¶ 62 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). Example III does not explicitly state that a hybridization temperature of about 42°C was employed in Example III. See ¶ 45 of attached §1.132 Declaration. Nonetheless, the evidence presented above demonstrates those of ordinary skill in the art of molecular biology would understand the hybridization temperature employed in Example III was also about 42°C. ¶ 62 of attached §1.132 Declaration (citing ¶¶ 59-61 of §1.132 Declaration).

We now further consider the hybridization temperature of about 42°C those of ordinary skill in the art of molecular biology would understand was employed in Example III. Use of a hybridization temperature of about 42°C in combination with 50% formamide in the hybridization solution is known to those of ordinary skill in the art of molecular biology as generally sufficient to establish stringent conditions:

> Membranes were hybridized either under conditions of moderate stringency [hybridization in 35% formamide, 0.1% Na-pyrophosphate, 50 mM Tris··Cl

> (pH 7.5), 5× SSC, 1% SDS, 5× Denhardt's, 100 μg/ml calf thymus DNA at 42°C, washing in 1× SSC/1% SDS at 60°C] or high stringency (hybridization in the same buffer except that the formamide concentration was 50% and at the same temperature . . . .

¶ 64 of attached §1.132 Declaration (citing page 11779 of Exhibit Q of §1.132 Declaration). Also see numbered para. 4 on the first page of Exhibit I, numbered para. 4 on the second page of Exhibit I, and numbered para. 4 on the third page of Exhibit I that each employ a hybridization temperature of about 42°C in combination with 50% formamide, where such use of 50% formamide is understood by those of ordinary skill in the art of molecular biology as evidence stringent hybridization conditions were intended and employed. ¶ 64 of attached §1.132 Declaration (citing Exhibit I, page G58 of Exhibit P, and page 11779 of Exhibit Q of §1.132 Declaration). The foregoing evidence regarding use of a hybridization temperature of about 42°C in combination with 50% formamide in the hybridization solution during the hybridization trial of Example III of the present application clearly illustrates, to those of ordinary skill in the art of molecular biology, use of stringent hybridization conditions during the hybridization trial of Example III. ¶ 65 of attached §1.132 Declaration (citing ¶¶ 58-64 of §1.132 Declaration).

Next, we consider the salmon sperm concentration of 100 μg/ml employed in the hybridization solution of Example III. ¶ 66 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). Persons of ordinary skill in the art of molecular biology also know another technique for improving specificity of base pairing entails incorporation of non-specific DNA in the hybridization solution. ¶ 67 of attached §1.132 Declaration (citing ¶ 17 and Exhibit E of §1.132 Declaration). The non-specific DNA is said to prevent the probe from hybridizing nonspecifically - that is with nucleic acid that is not complementary (specific) to the probe nucleic acid. ¶ 67 of attached §1.132 Declaration (citing ¶ 17 and Exhibit F of §1.132 Declaration).

As an example, salmon sperm at a concentration of 100 μg/ml is incorporated by those skilled in the art of molecular biology to complement and support high stringency hybridization by limiting non-specific nucleic acid binding during hybridization. ¶ 68 of attached §1.132 Declaration (citing Exhibit L of §1.132 Declaration). The evidence provided above regarding use of a hybridization

solution with a salmon sperm concentration of 100 μg/ml during the hybridization trial of Example III of the present application illustrates, to those of ordinary skill in the art of molecular biology, use of conditions that inhibit non-specific probe hybridization and therefore further support attainment of results consistent with use of stringent conditions during the hybridization trial of Example III. ¶ 69 of attached §1.132 Declaration.

We next consider the post-hybridization washing conditions of Example III that concluded with a final wash containing "0.1 x SSC, 0.1% SDS" at 60°C for 30 minutes. ¶ 70 of attached §1.132 Declaration (citing ¶ 45 of §1.132 Declaration). Beyond controlling the hybridization stringency directly, by varying the temperature at which hybridization is performed and/or the chemical composition of the hybridization solution used during hybridization experiments, those of ordinary skill in the art of molecular biology know hybridization stringency may also be controlled by post-hybridization washing conditions, such as the temperature and/or the salt concentration of the wash solution. ¶ 71 of attached §1.132 Declaration (citing ¶ 19 of §1.132 Declaration). For example:

> You can also control for stringency of hybridization at the next step, which is washing the unbound probe away from the blot. If you wash at high temperature or low salt concentration, you will remove any hybrids that are not perfectly matched.

¶ 71 of attached §1.132 Declaration (citing Exhibit H of §1.132 Declaration).

In this regard, those of ordinary skill in the art recognize that high stringency washing may be accomplished using a temperature at least as high as the hybridization temperature in combination with the final washing solution of Example III that contained 0.1x SSC and 0.1% SDS:

> Following hybridisation, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridisation temperature in 0.2-0.1 times SSC, 0.1% SDS.

¶ 72 of attached §1.132 Declaration (citing ¶ 0188 of Exhibit M of §1.132 Declaration). The foregoing evidence demonstrates those of ordinary skill in the art of molecular biology recognize high stringency washing may be accomplished using a final washing solution containing 0.1x SSC and 0.1% SDS, as was employed in Example III. ¶ 73 of attached §1.132 Declaration (citing ¶ 72 of §1.132 Declaration). Recognizing that increased temperature generally increases stringency, the foregoing evidence further

demonstrates those of ordinary skill in the art of molecular biology recognize high stringency washing may be accomplished at a temperature at least as high as the hybridization temperature; in Example III, the wash was accomplished at 60°C, which is at least as high as the about 42°C hybridization temperature employed in Example III. ¶ 73 of attached §1.132 Declaration (citing ¶¶ 13, 45, 62, and 72 and Exhibit C of §1.132 Declaration). Thus, use of high stringency washing, as employed in Example III of the above-identified application, illustrates, to those of ordinary skill in the art of molecular biology, use of conditions that further support attainment of results consistent with use of stringent conditions during the hybridization trial of Example III.

Example III of the above-identified application provides particular guidance about hybridization conditions that may be employed when practicing the hybridization methods of the present invention. ¶ 44 of attached §1.132 Declaration. This guidance of Example III illustrates that stringent hybridization conditions are suitable for practicing the hybridization methods of the present invention. ¶ 44 of attached §1.132 Declaration. Furthermore, this guidance of Example III teaches and discloses use of stringent hybridization conditions for practicing the hybridization methods of the present invention.

For instance, Example III employs a hybridization solution with a salt concentration of about 0.82 M sodium ion that those of ordinary skill in the art of molecular biology would recognize as a stringent hybridization condition. Also, those of ordinary skill in the art of molecular biology would recognize the Example III hybridization for twenty-one hours as being consistent with stringent hybridization conditions. Next, those of ordinary skill in the art of molecular biology would understand that a hybridization temperature of about 42°C was employed in Example III in combination with 50% formamide in the hybridization solution. Those of ordinary skill in the art of molecular biology would understand such use of a hybridization temperature of about 42°C in combination with 50% formamide in the hybridization solution would generally be sufficient to establish stringent conditions during the Example III hybridization.

Continuing, those of ordinary skill in the art of molecular biology would recognize the use in Example III of a hybridization solution with a salmon sperm concentration of 100 μg/ml as being consistent with use of stringent hybridization conditions. Finally, those of ordinary skill in the art of

molecular biology would recognize the use in Example III of stringent washing conditions (final washing solution with a temperature greater than the hybridization temperature and containing 0.1X SSC and 0.1% SDS), as use of conditions that may be part of an overall stringent hybridization protocol that support attainment of results consistent with use of stringent conditions during the Example III hybridization trial.

The guidance of Example III regarding hybridization conditions illustrates that stringent hybridization conditions are suitable for practicing the hybridization methods of the present invention. Indeed, the evidence provided herein illustrates one of ordinary skill in the art of molecular biology would recognize the hybridization conditions employed in Example III of the above-identified application constitute stringent hybridization conditions that would typically enable a probe, such as the bovine leptin cDNA molecule employed in Example III, to hybridize to a perfect or near perfect nucleic acid molecule complement (bovine leptin DNA) of the probe. ¶ 74 of attached §1.132 Declaration (citing ¶¶ 44-73 of the §1.132 Declaration, as supplemented by the Exhibits referenced in ¶¶ 44-73 of the §1.132 Declaration).

As noted above, in one aspect, the invention of the above-identified application is directed to a variety of nucleic acid molecules (and functional variants thereof) that encode bovine adipocyte polypeptide leptin and are capable of hybridizing to nucleotide sequences (and portions thereof). ¶ 75 of attached §1.132 Declaration (citing ¶¶ 20-22 of the §1.132 Declaration). In another aspect, the invention of the above-identified application is directed to hybridizing the nucleic acid molecules (and functional variants thereof) to nucleotide sequences (and portions thereof), such as various nucleotide sequences (and portions thereof) disclosed in the above-identified application. ¶ 75 of attached §1.132 Declaration (citing ¶¶ 20-22 of the §1.132 Declaration). The present application, primarily via Examples II and III, discloses conditions for hybridizing the nucleic acid molecules addressed in the present application. ¶ 76 of attached §1.132 Declaration.

As explained above, one of ordinary skill in the art of molecular biology, upon reviewing the hybridization conditions employed in Examples II and III of the above-identified application, would recognize these hybridization conditions as being stringent hybridization conditions that would typically enable a probe, such as the bovine leptin cDNA molecule employed in Examples II and III, to hybridize

to a perfect or near perfect nucleic acid molecule complement of the probe, such as bovine leptin mRNA or bovine leptin DNA. ¶ 76 of attached §1.132 Declaration. Consequently, since the above-identified application primarily discloses to one of ordinary skill in the art of molecular biology use of stringent hybridization conditions for hybridizing the nucleic acid molecules addressed in the present application, it is evident use of stringent hybridization conditions for hybridizing the nucleic acid molecules addressed in the present application is in fact disclosed and described in the present application. ¶ 77 of attached §1.132 Declaration.

Claims 13-30 are believed allowable. Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of claims 13-30 under the second paragraph of 35 U.S.C. §112 and that claims 13-30 be allowed.

Claim Rejection Under 35 U.S.C. §102(a) Based on the Tellam Submission

In the Office Action, the Examiner rejected claims 5 and 25-30 under 35 U.S.C. 102(a) as allegedly being anticipated by Genbank ACC. No. U43943, Bos taurus OBESE mRNA submission dated January 1996, hereinafter referred to as the "Tellam submission." According to the Examiner:

> TELLAM et al. disclose a nucleic acid molecule (mRNA) which is an allelic variant of SEQ ID NO:3 of the instant application. The nucleotide sequence differs from that of SEQ ID NO:3 in length (the prior art is longer) and differs in sequence at 14 positions. This translates into 2 amino acid differences (see bolded amino acids in the attached reference) and 18 additional amino acids at the N-terminus of the protein, which could be leader sequence. Therefore, the instant claims are anticipated by the prior art.
>
> Applicant asserts that Exhibit D (Declaration under 37 C.F.R. 1.131) is sufficient to overcome the instant rejection. This argument is not persuasive and the Declaration is not effective for the following reasons. MPEP 715.03 (B) states where the only pertinent disclosure in the reference is a single species of the claimed genus, the applicant can overcome the rejection directly under 37 C.F.R. 1.131 by showing prior possession of the species disclosed in the reference. Applicant has not shown prior possession of the species disclosed in the reference. MPEP 715.03 (B) continues with proof of prior completion of a species

> different from the species of the reference will be sufficient to overcome a reference indirectly under 37 C.F.R. 1.131 if the species shown in the reference would have been obvious in view of the species shown to have been made by the application. However, the species in the reference would not have been obvious in view of the species in the instant application, absent evidence to the contrary in and view of current case law governing biotech applications. Alternatively, the applicant may be able to antedate the reference indirectly by, for example, showing prior completion of one or more species which put him or her in possession of the claimed genus prior to the reference's date. Applicant has not done this. Therefore, the rejection is maintained.

Despite the Examiner's comments, the Tellam submission does not anticipate any of claims 23-30. As noted above, claim 5 has been canceled.

First, Applicant notes claim 23 depends from independent claim 22 that the Examiner has not rejected based on the Tellam submission. Claim 22 is believed allowable, and claim 23 that depends from independent claim 22 is likewise also believed allowable.

Next, claim 24 of the above-identified application reads as follows:

> *24. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, the DNA molecule consisting of the nucleotide sequence SEQ ID NO:3 or a variant thereof, wherein the DNA molecule or the variant thereof hybridizes to substantially all of the nucleotide sequence of SEQ ID NO:3 when placed in contact with the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.*

Claim 24 thus defines an isolated single or double-stranded DNA molecule. The Tellam submission discloses a mRNA molecule, as the Examiner notes, but does not disclose any DNA molecules. Since the Tellam submission does not disclose any DNA molecules, it is clear the Tellam submission does not disclose the isolated single or double-stranded DNA molecule defined in claim 24. And therefore does not anticipate claim 24. Claim 24 is therefore believed allowable despite the Examiner's rejection of claim 24 based on the Tellam submission.

We next consider independent claims 25 and 27-30. Claim 25 reads as follows:

> *25. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, the mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3 or a variant thereof, wherein the mRNA molecule or the variant of the*

mRNA molecule hybridizes to the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Next, claim 27 reads as follows:

27. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, the mRNA molecule encoded by a nucleotide sequence SEQ ID NO:3 or a functional derivative thereof, wherein the functional derivative of the isolated mRNA molecule hybridizes to substantially all of the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Next, claim 28 reads as follows:

28. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, the mRNA molecule encoded by a nucleotide sequence SEQ ID NO:3 or a functional variant thereof, wherein the functional variant hybridizes to substantially all of the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Continuing, claim 29 reads as follows:

29. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, wherein the isolated mRNA molecule hybridizes to substantially all of an mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Finally, claim 30 reads as follows:

30. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, wherein the isolated mRNA molecule hybridizes to an mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Thus, claims 25-30 define, in various ways, an isolated mRNA molecule which encodes *a bovine adipocyte polypeptide leptin*.

In the Office Action, the Examiner alleged that Applicant's Declaration under 37 C.F.R. 1.131 submitted in reply to the prior Office Action did not show "prior completion of one or more species

which put him or her in possession of the claimed genus prior to the reference's date." The Examiner provided no basis for this allegation and provided no explanation as to why the Examiner felt Applicant's Declaration under 37 C.F.R. 1.131 was insufficient, in contravention of MPEP §716.01. Specifically, if the Examiner does not find the factual and opinion evidence provided in the Declarations to be convincing, the Examiner must "specifically explain why the evidence is insufficient." (MPEP §716.01). The Examiner cannot discharge this obligation of explaining any insufficiency of the Declarations using only one or two short and general statements or simply by saying the requirements of the Patent Office were not met." Applicant therefore respectfully requests clarification and explanation about why the Examiner determined Applicant's Declaration under 37 C.F.R. 1.131 submitted in response to the prior Office Action was insufficient.

Furthermore, Applicant's note the Examiner relies on MPEP §715.03 (B) that concerns genus claims. The Examiner apparently asserts that claims 23-30 are genus claims. However, the Examiner does not provide any rationale, support, or basis for characterizing claims 23-30 as genus claims. In essence, the Examiner states a conclusion without providing any evidence or authority in support of that conclusion.

Despite the Examiner's characterization of claims 23-30 as genus claims, Applicant asserts claims 23-30 should instead be viewed as claims that recite a combination and sub-combination. When properly characterized, claims 23-30 should instead be evaluated using MPEP §715.03 (A) or MPEP §715.03 (C), rather than MPEP §715.03 (B). With this understanding, Applicant's showing, in Applicant's Declaration under 37 C.F.R. 1.131 submitted in response to the prior Office Action, was actually sufficient to define over the Tellam reference.

In this regard, we focus on the independent claims. For example, the preamble of claim 24 recites a combination ("An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin"), and the body of claim 24 recites alternative subcombinations ("SEQ ID NO:3 or a variant thereof," which are recited, alternative, components or fragments of the combination, namely the isolated DNA molecule that encodes the bovine leptin). Thus claim 24 recites a pair of alternative subcombinations. Though not important for characterizing the claim form, claim 24 further

recites other features, beyond the alternative subcombination features detailed above. Though the claim terminology varies, analogous reasoning to that provided above in regard to claim 24 applies to independent claims 25, 27, and 28; to claims 23-24 that depend from independent claim 22; and to claim 26 that depends from independent claim 25.

As another example, the preamble of claim 30 recites a combination ("An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin"), and the body of claim 30 recites a subcombination, namely a requirement that "the isolated mRNA molecule hybridizes to an mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3." Thus, claim 30 recites a complement to SEQ ID NO:3, where the complement includes at least one component or fragment of the combination, namely the isolated mRNA molecule that encodes the bovine leptin, and where the complement allows and supports hybridization of the isolated mRNA molecule "to an mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3" under the environment recited in claim 30. Thus claim 24 recites a subcombination. Though the claim terminology varies, analogous reasoning to that provided above in regard to claim 30 applies to independent claim 30.

Applicant has attached a Declaration under 37 C.F.R. §1.131 that Dr. Michael Spurlock, the sole inventor named in the above-identified application, has executed. The attached Declaration under 37 C.F.R. §1.131 contains facts that establish completion of the invention, as defined in claims 23-30, on or before December 26, 1995, which is a date prior to the December 27, 1995 effective date of the Tellam submission. The attached Declaration under 37 C.F.R. §1.131 is identified as Exhibit E of this Amendment After Final.

Dr. Spurlock's Declaration under 37 C.F.R. §1.131 describes the isolation and sequencing of at least two copies of bovine leptin cDNA clones under Dr. Spurlock's direction. ¶¶ 9.D.i.-vii. of attached §1.131 Declaration. Under Dr. Spurlock's directions, these bovine leptin cDNA clones were submitted to National Bioscience, Inc., (a commercial laboratory skilled in the protocol of performing gene sequencing) for sequencing. ¶ 9.E. of attached §1.131 Declaration. National Bioscience, Inc., reported that one of the bovine leptin cDNA clones had the nucleotide sequence of SEQ ID NO:3 of U.S. Patent Application No. 08/688,908 (now U.S. Patent No. 6,297,027). ¶ 9.F. of attached §1.131

Declaration. The nucleotide sequence of SEQ ID NO:3 of U.S. Patent Application No. 08/688,908 is also disclosed in the above-identified application as the nucleotide sequence of SEQ ID NO:3. The above-identified application is a continuation-in part application of, and claims priority from, U.S. Patent Application No. 08/688,908. ¶ 9.F. of the attached §1.131 Declaration.

The sequence of the second bovine leptin cDNA clone that contained bovine leptin cDNA was confirmed when Brian Hoffman of National Biosciences, Inc. sent Dr. Ji a letter (attached as Exhibit A of the attached §1.131 Declaration) in response to Dr. Ji's instructions with an enclosed paper (attached as Exhibit B of the attached §1.131 Declaration). ¶ 9.G. of the attached §1.131 Declaration. The second bovine leptin cDNA clone included the sequence of a 450 base bovine leptin cDNA clone that Dr. Spurlock characterizes as a functional derivative, functional variant, or variant of bovine leptin DNA encoding the nucleotide sequence of SEQ ID NO:3. ¶ 9.H. of the attached §1.131 Declaration. The nucleotide sequence of the 450 bovine leptin cDNA base clone differs (see ¶¶ 9.H.i.-xi of the attached §1.131 Declaration) from the nucleotide sequence of SEQ ID NO:3,which prompted Dr. Spurlock's characterization of the 450 bovine leptin cDNA base clone as a functional derivative, functional variant, or variant.

Though the nucleotide sequence of the 450 bovine leptin cDNA base clone is characterized as a functional derivative, a functional variant, and a variant of the nucleotide sequence of SEQ ID NO:3, the nucleotide sequence of the 450 bovine leptin cDNA base clone differs from the nucleotide sequence of SEQ ID NO:3 at only a very limited number of nucleotide positions. ¶¶ 9.H.i.-xi of the attached §1.131 Declaration. Therefore, the nucleotide sequence of the 450 bovine leptin cDNA base clone and the nucleotide sequence of SEQ ID NO:3 are said to exhibit only very little nucleotide mismatching. (See Exhibits B and C of this Amendment After Final). Consequently, upon hybridization under stringent hybridization conditions, those of ordinary skill in the art of microbiology would understand base-pairing sufficient to allow hybridization of the 450 bovine leptin cDNA base clone and the nucleotide sequence of SEQ ID NO:3 and consequent formation of a hybrid molecule containing the nucleotide sequence of the 450 bovine leptin cDNA base clone and the nucleotide sequence of SEQ ID NO:3 would occur. (See Exhibits B and C of this Amendment After Final).

Returning to the claims, claim 22 defines an isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, where the DNA molecule may consist of a functional derivative of the nucleotide sequence SEQ ID NO:3 that hybridizes to the nucleotide sequence of SEQ ID NO:3 when placed in contact with the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, and ¶9.H. of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 22. ¶¶ 9.J.-K. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration may be the functional derivative of the nucleotide sequence SEQ ID NO:3 defined in claim 22. ¶ 9.K. of the attached §1.131 Declaration. As noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the 450 bovine leptin cDNA base clone (functional derivative of claim 22) and the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 24 defines an isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, where the DNA molecule may consist of a variant of the nucleotide sequence SEQ ID NO:3 that hybridizes to the nucleotide sequence of SEQ ID NO:3 when placed in contact with the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, and ¶9.H. of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 24. ¶¶ 9.L.-M. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration may be the variant functional derivative of the nucleotide sequence SEQ ID NO:3 defined in claim 24. ¶ 9.M. of the attached §1.131 Declaration. As noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the 450 bovine leptin cDNA base clone (variant of claim 24) and the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 25 defines an isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, where the isolated mRNA molecule may be a variant of an mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 and hybridizes to the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, ¶9.H., ¶10, and ¶11 of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 25. ¶¶ 12.-13. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration is the complement of an mRNA molecule that in turn is the variant defined in claim 25 in terms of the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3. ¶ 13. of the attached §1.131 Declaration. Analogous to the comments noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization a variant of an mRNA molecule encoded by the 450 bovine leptin cDNA base clone (variant of claim 25) and an mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 27 defines an isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, where the isolated mRNA molecule may be a functional derivative of an mRNA molecule that is encoded by the nucleotide sequence SEQ ID NO:3 and hybridizes to the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 when placed in contact with the mRNA molecule that is encoded by the nucleotide sequence SEQ ID NO:3 under stringent hybridizing conditions.. The facts and evidence presented in ¶9.E, ¶9.G, ¶9.H., ¶10, and ¶11 of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 27. ¶¶ 14.-15. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration is the complement of an mRNA molecule that in turn is the functional derivative defined in claim 27 in terms of the mRNA molecule encoded by the

nucleotide sequence SEQ ID NO:3. ¶ 15. of the attached §1.131 Declaration. Analogous to the comments noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of an mRNA molecule encoded by the 450 bovine leptin cDNA base clone (functional derivative of claim 27) and an mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 28 defines an isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, where the isolated mRNA molecule may be a functional variant of an mRNA molecule that is encoded by the nucleotide sequence SEQ ID NO:3 and hybridizes to the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 when placed in contact with the mRNA molecule that is encoded by the nucleotide sequence SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, ¶9.H., ¶10, and ¶11 of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 29. ¶¶ 16.-17. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration is the complement of an mRNA molecule that in turn is the functional variant defined in claim 28 in terms of the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3. ¶ 17. of the attached §1.131 Declaration. Analogous to the comments noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of an mRNA molecule encoded by the 450 bovine leptin cDNA base clone (functional variant of claim 28) and an mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 29 defines an isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, where the isolated mRNA molecule hybridizes to substantially all of an mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, ¶9.H., ¶10, and ¶11 of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed

(reduced to practice) the invention of the above-identified application, as defined in claim 29. ¶¶ 18.-19. of the attached §1.131 Declaration. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration is the complement of the mRNA molecule defined in claim 29 as substantially hybridizing to the mRNA molecule encoded by the nucleotide sequence SEQ ID NO:3 under stringent hybridizing conditions. ¶ 19. of the attached §1.131 Declaration. Analogous to the comments noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the isolated bovine leptin cDNA clone (i.e. the 450 bovine leptin cDNA base clone mentioned in ¶9.G and ¶9.H. of the attached §1.131 Declaration) that is the complement of the mRNA molecule defined in claim 29 to substantially all of an mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions. Analogous comments to those provided above illustrate Dr. Spurlock's completion (reduction to practice) of the invention of the above-identified application, as defined in claim 30. ¶¶ 20.-21. of the attached §1.131 Declaration.

Because the present invention, as defined in claims 24, 25, and 27-30, was completed on or before December 26, 1995 and therefore prior to the effective date of the Tellam submission, the Tellam submission does not anticipate the invention of the above-identified application, as defined in claims 24, 25, and 27-30. Also, as explained above, the Tellam submission does not disclose any DNA molecules and consequently does not disclose the isolated single or double-stranded DNA molecule defined in claim 24.

Claims 22, 24, 25, and 27-30 are believed allowable. Claim 23 is also believed allowable, since claim 23 depends from allowable claim 22. Also, claim 26 is believed allowable, since claim 26 depends from allowable claim 25. Consequently, Applicant respectfully requests that the Examiner reconsider and withdraw the rejections of claims 23-30 under 35 U.S.C.§102(a) based on the Tellam submission and that claims 23-30 be allowed.

Claim Rejections Under 35 U.S.C. §103(a) Based on the Tellam Submission

In the Office Action, the Examiner rejected claims 1-4 and 13-24 under 35 U.S.C. 103(a) as allegedly being obvious in light of the Tellam submission. According to the Examiner:

> TELLAM et al. disclose a nucleic acid molecule (mRNA) which is an allelic variant of SEQ ID NO:3 of the instant application. TELLAM et al. do not disclose single or double stranded DNA, an expression vector or plasmic comprising the DNA or a host cell transformed or transfected with the plasmid. However, at the time of the instant invention, it would have been *prima facie* obvious to one of ordinary skill in the art to use the mRNA molecule of TELLAM et al. to generate a DNA molecule, which could then be placed into an expression vector or plasmid, and then placed into a host cell for the purpose of propagating the nucleic acid, as well as for expression of the encoded protein of the nucleic acid of TELLAM et al. One would be motivated to do this because TELLAM et al. identify the nucleic acid as encoding bovine obesity protein (a.k.a. leptin) and this protein is known to be valuable in regulation of weight in mammals. At the time of the instant invention, such methods and techniques were old and well-known in the art, as evidenced by the disclosure of the instant specification, at pages 9-10, therefore, a reasonable expectation of success was also present.
>
> Applicant asserts that the Declaration (Exhibit D and/or F) filed under 1.131 obviates the instant rejection. This argument is not persuasive and the Declaration is ineffective for the reasons provided above. Therefore, the rejection is maintained.

As noted above, claims 1-4 have been canceled. Despite the Examiner's comments, the Tellam submission does not teach, suggest, disclose, or render obvious the invention of the above-identified application, as defined in claims 13-24.

In the Office Action, the Examiner, by reference to the Examiner's comments with regard to the §102 rejection based on the Tellam submission, alleged that Applicant's Declaration under 37 C.F.R. 1.131 submitted in reply to the prior Office Action did not show "prior completion of one or more species which put him or her in possession of the claimed genus prior to the reference's date." The Examiner provided no basis for this allegation and provided no explanation as to why the Examiner felt Applicant's Declaration under 37 C.F.R. 1.131 was insufficient, in contravention of MPEP §716.01.

Specifically, if the Examiner does not find the factual and opinion evidence provided in the Declarations to be convincing, the Examiner must "specifically explain why the evidence is insufficient." (MPEP §716.01). The Examiner cannot discharge this obligation of explaining any insufficiency of the Declarations using only one or two short and general statements or simply by saying the requirements of the Patent Office were not met." Applicant therefore respectfully requests clarification and explanation about why the Examiner determined Applicant's Declaration under 37 C.F.R. 1.131 submitted in response to the prior Office Action was insufficient.

Furthermore, as noted above, the Examiner has apparently characterized claims 13-24 as genus claims. However, the Examiner does not provide any rationale, support, or basis for characterizing claims 13-24 as genus claims. In essence, the Examiner states a conclusion without providing any evidence or authority in support of that conclusion. As noted above, Applicant asserts claims 23-24 should instead be viewed as claims that recite a combination and sub-combination. For reasons analogous to those provided above in response to the Examiner's rejection under §102 rejection based on the Tellam submission, Applicant asserts claims 13-22 should instead be viewed as claims that recite a combination and sub-combination. Thus, when properly characterized, claims 13-24 should instead be evaluated using MPEP §715.03 (A) or MPEP §715.03 (C), rather than MPEP §715.03 (B).

As an initial matter, the Examiner has failed to state a prima facie case for the alleged obviousness of attaining the invention of the above-identified application, as defined in claims 13-24, based on the very limited disclosure of the Tellam submission. According to the Examiner, obvious to one of ordinary skill in the art to use the mRNA molecule of TELLAM et al. to generate a DNA molecule. However, the mere disclosure of the mRNA molecule in Tellam in accordance with the Examiner's comments does not teach or suggest the Examiner's alleged transformation to the complementary cDNA molecule. That teaching or suggestion must necessarily come from outside of the Tellam submission and apparently came by virtue of hindsight reconstruction using the above-identified application as a road map. Such hindsight reconstruction is never an appropriate basis for alleging obviousness.

Applicant's attached Declaration under 37 C.F.R. §1.131 (as Exhibit E of this Amendment After Final) by Dr. Michael Spurlock, the sole inventor named in the above-identified application, was previously noted and discussed above in relation to the Examiner's §102 rejection based on the Tellam submission. The attached Declaration under 37 C.F.R. §1.131 contains facts that establish completion of the invention, as defined in claims 13-24, on or before December 26, 1995, which is a date prior to the December 27, 1995 effective date of the Tellam submission. For the sake of efficiency, Applicant's comments regarding the Declaration under 37 C.F.R. §1.131 provided above in relation to the Examiner's §102 rejection based on the Tellam submission are not repeated here.

As noted above in relation to the Examiner's §102 rejection based on the Tellam submission, the present invention, as defined in claim 22, was completed on or before December 26, 1995 and therefore prior to the effective date of the Tellam submission. Therefore, the Tellam submission does not render obvious the invention of the above-identified application, as defined in claim 22 and claim 22 is deemed allowable over the Examiner's rejection based on the Tellam submission.

Next, claim 13 defines an isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin and hybridizes to the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.F, ¶9.G, and ¶9.H. of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 13. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration may be the isolated single or double-stranded DNA molecule defined in claim 13. As noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the 450 bovine leptin cDNA base clone (the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration) and the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions. Alternatively, the isolated bovine leptin cDNA clone that is the subject of ¶9.F of the attached §1.131 Declaration may be the isolated single or double-stranded DNA molecule defined in claim 13. As noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the isolated bovine leptin cDNA base clone

(the subject of ¶9.F of the attached §1.131 Declaration) and the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

Next, claim 21 defines an isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, where the DNA molecule may consist of a functional variant of the nucleotide sequence SEQ ID NO:3 that is capable of hybridizing to substantially all of the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions. The facts and evidence presented in ¶9.E, ¶9.G, and ¶9.H. of the attached §1.131 Declaration demonstrate that, during the period ending on or before December 26, 1995, Dr. Spurlock completed (reduced to practice) the invention of the above-identified application, as defined in claim 21. The isolated bovine leptin cDNA clone that is the subject of ¶9.G and ¶9.H. of the attached §1.131 Declaration may be the functional variant of the nucleotide sequence SEQ ID NO:3 defined in claim 21. As noted above, those of ordinary skill in the art of microbiology understand base-pairing sufficient to allow hybridization of the 450 bovine leptin cDNA base clone (functional variant of claim 21) and the nucleotide sequence of SEQ ID NO:3 would occur under stringent hybridization conditions.

The present invention, as defined in claims 13, 21, and 22, was completed on or before December 26, 1995, and therefore prior to the effective date of the Tellam submission. Consequently, the Tellam submission does not render obvious the invention of the above-identified application, as defined in claims 13, 21, and 22.

Claims 13, 21, and 22 are believed allowable. Claims 14-20 are also believed allowable, since claims 14-20 each depend from allowable claim 13. Likewise, claim 23 is believed allowable, since claim 23 depends from allowable claim 22. Consequently, Applicant respectfully requests that the Examiner reconsider and withdraw the rejections of claims 13-23 under 35 U.S.C.§103(a) based on the Tellam submission and that claims 13-23 be allowed.

Claim Rejections Under 35 U.S.C. §103(a) Based On The Friedman Patent

In the Office Action, the Examiner rejected claims 22 and 24-27 under 35 U.S.C. 103(a) as allegedly being unpatentable over U.S. Patent N. 6,309,853 to Friedman et al. (subsequently referred to as the "Friedman patent"). In support of this rejection, the Examiner stated:

> The instant claims are directed to isolated nucleic acids which encode bovine leptin and hybridize to SEQ ID NO:3 or a 'functional derivative thereof' (see claims 22, 27) or 'variant'(see claims 24-26). The prior art of Friedman et al. (U.S. Pat. No. 6,309,853) disclose nucleic acids which encode human and mouse leptin, which would be considered functional derivatives and/or variants of SEQ ID NO:3 since they encode leptin molecules and would possess similar functional properties as those of the bovine leptin, absent evidence to the contrary. Friedman et al. teach that the leptin gene (or OB) could be isolated from domestic animals using the methods disclosed therein (see column 26, line 53 to column 27, line 49). Friedman et al. specifically mention cattle as a domestic animal for which leptin would be useful (see column 48, lines 41-57). Friedman et al. do not specifically disclose an isolated nucleic acid encoding a bovine leptin polypeptide. However, it would have been obvious to use the nucleic acid of Friedman et al. encoding human or mouse leptin and hybridize it to a bovine DNA library and isolate a nucleic acid molecule encoding bovine leptin because Friedman et al. teach methods for isolating leptin encoding nucleic acids and also teach that it would be beneficial to administer leptin to cattle. Therefore, the invention as a whole would have been obvious at the time it was made, absent evidence to the contrary.
>
> Applicant should note that the instant rejection is being made because the claims do not require the specifics of SEQ ID NO:3, and therefore, methods of isolating nucleic acids for leptin using a functional equivalent of bovine leptin encoding DNA encompasses methods using human or murine DNA encoding leptin.

Despite the Examiner's allegations, the Friedman patent does not teach, suggest, disclose, or make obvious the invention of the above-identified application, as defined in claims 22 and 24-27.

Independent claim 22 reads as follows:

> *22. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, the DNA molecule consisting of the nucleotide sequence of SEQ ID NO:3 or a functional derivative thereof, wherein the*

DNA molecule or the functional derivative thereof hybridizes to the nucleotide sequence of SEQ ID NO:3 when placed in contact with the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Claim 22 thus defines an isolated bovine leptin DNA molecule that contains SEQ ID NO:3 or a functional derivative of SEQ ID NO:3 and further specifies the isolated bovine leptin DNA molecule or the functional derivative thereof hybridizes to SEQ ID NO:3 under stringent hybridization conditions.

Next, independent claim 24 reads as follows:

24. (Currently Amended) An isolated single or double-stranded DNA molecule which encodes a bovine adipocyte polypeptide leptin, the DNA molecule consisting of a nucleotide sequence of SEQ ID NO:3 or a variant thereof, wherein the DNA molecule or the variant thereof hybridizes to substantially all of the nucleotide sequence of SEQ ID NO:3 when placed in contact with the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Claim 24 thus defines an isolated bovine leptin DNA molecule that contains SEQ ID NO:3 or a variant of SEQ ID NO:3 and further specifies that the isolated bovine leptin DNA molecule or the variant thereof hybridizes to substantially all of SEQ ID NO:3 under stringent hybridization conditions.

Next, independent claim 25 reads as follows:

25. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, the mRNA molecule encoded by a nucleotide sequence of SEQ ID NO:3 or a variant of the mRNA molecule, wherein the mRNA molecule or the variant of the mRNA molecule hybridizes to the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Claim 25 thus defines an isolated bovine leptin mRNA molecule of SEQ ID NO:3 or a variant of SEQ ID NO:3 and further specifies that the isolated bovine leptin mRNA molecule or the variant thereof hybridizes to SEQ ID NO:3 under stringent hybridization conditions.

Finally, independent claim 27 reads as follows:

27. (Currently Amended) An isolated mRNA molecule which encodes a bovine adipocyte polypeptide leptin, the mRNA molecule encoded by a nucleotide sequence SEQ ID NO:3 or a functional derivative thereof, wherein the functional derivative of the isolated mRNA molecule hybridizes to substantially all of the mRNA molecule encoded by the nucleotide sequence of SEQ ID NO:3 when placed in contact with the mRNA

molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Hence, claim 27, defines a bovine leptin mRNA molecule of SEQ ID NO:3 or a functional derivative of SEQ ID NO:3 or a functional derivative of SEQ ID NO:3 and further specifies that the isolated bovine leptin mRNA molecule or the functional derivative thereof hybridizes to substantially all of SEQ ID NO:3 under stringent hybridization conditions.

As noted above, the Friedman patent does not teach, suggest, or disclose the invention of the above-identified application, as defined in claims 22 and 24-27. Consistent with the Examiner's observation, the Friedman patent does disclose murine and human leptin DNA sequences and polypeptides. ¶ 81 of attached §1.132 Declaration. Also, consistent with the Examiner's observation, the Friedman patent does not disclose any bovine leptin DNA (or mRNA) molecules or polypeptides. ¶ 82 of attached §1.132 Declaration. Furthermore, consistent withe the Examiner's observation, the Friedman patent does not disclose any functional derivative or variant DNA (or mRNA) molecules that encode for bovine leptin polypeptide. ¶ 83 of attached §1.132 Declaration.

Furthermore, the human and murine leptin DNA sequences disclosed in the Friedman patent differ substantially from the bovine leptin DNA sequences disclosed in the above-identified application; consequently, the bovine leptin of the present application that is based on the bovine leptin DNA sequences (and functional variants thereof) disclosed in the above-identified application differs in substantial detail from both the human leptin and the murine leptin disclosed in the Friedman patent. ¶ 84 of attached §1.132 Declaration. As a result, the bovine leptin of the present application is functionally different from both the human leptin and the murine leptin disclosed in the Friedman patent. ¶ 85 of attached §1.132 Declaration.

For example, when recombinant growth hormone is administered by injection to castrate male cattle, the castrate male cattle exhibit <u>increased</u> adipose tissue leptin mRNA expression. ¶ 86 of attached §1.132 Declaration (citing Exhibit T of the §1.132 Declaration). See also ¶¶ 89-98 of attached §1.132 Declaration. On the other hand, when recombinant growth hormone is administered by injection to male mice, the male mice exhibit <u>essentially no increased</u> adipose tissue leptin mRNA expression. ¶

87 of attached §1.132 Declaration (citing Exhibit U of the §1.132 Declaration). See also ¶¶ 100-111 of attached §1.132 Declaration.

Since recombinant growth hormone administration increases adipose tissue leptin mRNA expression in male cattle, while recombinant growth hormone administration causes essentially no increased adipose tissue leptin mRNA expression in male mice, the effects of growth hormone administration on leptin mRNA expression in male cattle versus in male mice differ dramatically, and it is consequently evident the bovine leptin protein is functionally very different from the rat leptin protein. ¶ 88 of attached §1.132 Declaration. Otherwise stated, based on the documented differences in adipose tissue leptin mRNA expression after administration to normal male cows (per the Spurlock publication) versus normal male rats (per the Lee publication), it is evident that bovine leptin surprisingly functions very differently after administration of growth hormone to male cattle as compared to how rat leptin functions after administration of growth hormone to male rats. ¶ 112 of attached §1.132 Declaration (citing ¶¶ 98-99 and 109-110 of the §1.132 Declaration).

As another example, when dexamethasone (a glucocorticoid) is administered to multiparous non-lactating Holstein cows, the dexamethasone administration fails to change plasma leptin protein levels. ¶ 113 of attached §1.132 Declaration (citing Exhibit V of the §1.132 Declaration). See also ¶¶ 116-120 of attached §1.132 Declaration. On the other hand, when dexamethasone is administered to healthy human volunteers, the healthy human volunteers exhibit significantly increased leptin expression in the serum after dexamethasone administration. ¶ 114 of attached §1.132 Declaration (citing Exhibit W of the §1.132 Declaration). See also ¶¶ 122-128 of attached §1.132 Declaration.

Since dexamethasone administration fails to change plasma leptin concentrations in cows, while dexamethasone administration significantly increased serum leptin levels in healthy human volunteers, it is evident bovine leptin based on the documented differences in bovine leptin protein expression after dexamethasone administration to normal cows (per the Spurlock publication) versus human leptin protein expression after dexamethasone administration to healthy human volunteers (per the Fried publication), it is evident that bovine leptin protein surprisingly functions very differently from

molecule encoded by the nucleotide sequence of SEQ ID NO:3 under stringent hybridizing conditions.

Hence, claim 27, defines a bovine leptin mRNA molecule of SEQ ID NO:3 or a functional derivative of SEQ ID NO:3 or a functional derivative of SEQ ID NO:3 and further specifies that the isolated bovine leptin mRNA molecule or the functional derivative thereof hybridizes to substantially all of SEQ ID NO:3 under stringent hybridization conditions.

As noted above, the Friedman patent does not teach, suggest, or disclose the invention of the above-identified application, as defined in claims 22 and 24-27. Consistent with the Examiner's observation, the Friedman patent does disclose murine and human leptin DNA sequences and polypeptides. ¶ 81 of attached §1.132 Declaration. Also, consistent with the Examiner's observation, the Friedman patent does not disclose any bovine leptin DNA (or mRNA) molecules or polypeptides. ¶ 82 of attached §1.132 Declaration. Furthermore, consistent withe the Examiner's observation, the Friedman patent does not disclose any functional derivative or variant DNA (or mRNA) molecules that encode for bovine leptin polypeptide. ¶ 83 of attached §1.132 Declaration.

Furthermore, the human and murine leptin DNA sequences disclosed in the Friedman patent differ substantially from the bovine leptin DNA sequences disclosed in the above-identified application; consequently, the bovine leptin of the present application that is based on the bovine leptin DNA sequences (and functional variants thereof) disclosed in the above-identified application differs in substantial detail from both the human leptin and the murine leptin disclosed in the Friedman patent. ¶ 84 of attached §1.132 Declaration. As a result, the bovine leptin of the present application is functionally different from both the human leptin and the murine leptin disclosed in the Friedman patent. ¶ 85 of attached §1.132 Declaration.

For example, when recombinant growth hormone is administered by injection to castrate male cattle, the castrate male cattle exhibit <u>increased</u> adipose tissue leptin mRNA expression. ¶ 86 of attached §1.132 Declaration (citing Exhibit T of the §1.132 Declaration). See also ¶¶ 89-98 of attached §1.132 Declaration. On the other hand, when recombinant growth hormone is administered by injection to male mice, the male mice exhibit <u>essentially no increased</u> adipose tissue leptin mRNA expression. ¶

87 of attached §1.132 Declaration (citing Exhibit U of the §1.132 Declaration). See also ¶¶ 100-111 of attached §1.132 Declaration.

Since recombinant growth hormone administration increases adipose tissue leptin mRNA expression in male cattle, while recombinant growth hormone administration causes essentially no increased adipose tissue leptin mRNA expression in male mice, the effects of growth hormone administration on leptin mRNA expression in male cattle versus in male mice differ dramatically, and it is consequently evident the bovine leptin protein is functionally very different from the rat leptin protein. ¶ 88 of attached §1.132 Declaration. Otherwise stated, based on the documented differences in adipose tissue leptin mRNA expression after administration to normal male cows (per the Spurlock publication) versus normal male rats (per the Lee publication), it is evident that bovine leptin surprisingly functions very differently after administration of growth hormone to male cattle as compared to how rat leptin functions after administration of growth hormone to male rats. ¶ 112 of attached §1.132 Declaration (citing ¶¶ 98-99 and 109-110 of the §1.132 Declaration).

As another example, when dexamethasone (a glucocorticoid) is administered to multiparous non-lactating Holstein cows, the dexamethasone administration fails to change plasma leptin protein levels. ¶ 113 of attached §1.132 Declaration (citing Exhibit V of the §1.132 Declaration). See also ¶¶ 116-120 of attached §1.132 Declaration. On the other hand, when dexamethasone is administered to healthy human volunteers, the healthy human volunteers exhibit significantly increased leptin expression in the serum after dexamethasone administration. ¶ 114 of attached §1.132 Declaration (citing Exhibit W of the §1.132 Declaration). See also ¶¶ 122-128 of attached §1.132 Declaration.

Since dexamethasone administration fails to change plasma leptin concentrations in cows, while dexamethasone administration significantly increased serum leptin levels in healthy human volunteers, it is evident bovine leptin based on the documented differences in bovine leptin protein expression after dexamethasone administration to normal cows (per the Spurlock publication) versus human leptin protein expression after dexamethasone administration to healthy human volunteers (per the Fried publication), it is evident that bovine leptin protein surprisingly functions very differently from

human leptin protein. ¶ 129 of attached §1.132 Declaration (citing ¶¶ 113-115, 120-121 and 127-128. of the §1.132 Declaration).

The foregoing factual evidence illustrates the human leptin disclosed in the Friedman patent does not, despite the Examiner's contentions to the contrary (see ¶ 78 of attached §1.132 Declaration), necessarily, or actually, possess functional properties similar to the functional properties of the bovine leptin disclosed in the above-identified application. ¶ 130 of attached §1.132 Declaration. For example, based on the documented differences in adipose tissue leptin mRNA expression after growth hormone administration to male cows (per the Spurlock publication) versus adipose tissue leptin mRNA expression after growth hormone administration to male rats (per the Lee publication), it is evident bovine leptin protein surprisingly functions very differently as compared to how rat leptin protein functions. ¶ 131 of attached §1.132 Declaration (citing ¶¶ 86-88, 98-99, and 109-111 of the §1.132 Declaration).

The documented differences in leptin mRNA expression after growth hormone administration to normal cows (per the Spurlock publication) versus normal rats (per the Lee publication) are surprising and unexpected, since there is no evidence of record, such as in the Spurlock publication, the Lee publication, or the Friedman patent, that would suggest the differential effects on leptin mRNA expression caused by growth hormone administration in male cows versus growth hormone administration in male rats. ¶ 132 of attached §1.132 Declaration. Furthermore, the documented differences in leptin mRNA expression after growth hormone administration to normal cows (per the Spurlock publication) versus growth hormone administration to normal rats (per the Lee publication) are surprising and unexpected, since there is no evidence of record, such as in the Spurlock publication, the Lee publication, or the Friedman patent, that would suggest growth hormone administration in rats would have essentially no effect on leptin mRNA expression by the rats, while growth hormone administration in cows would cause an increase in leptin mRNA expression by the cows. ¶ 133 of the attached §1.132 Declaration.

Therefore, based on the factual results noted above in Paragraphs 130-133 and despite the Examiner's contentions to the contrary (see ¶ 78 of the attached §1.132 Declaration), the Examiner's speculative suggested hybridization of the nucleic acid of the Friedman patent that encodes murine leptin

to a bovine DNA library and subsequent isolation of a nucleic acid molecule encoding bovine leptin is not suggested. ¶ 134 of attached §1.132 Declaration (citing ¶¶ 130-133 of attached §1.132 Declaration). since the functional characteristics of the murine leptin disclosed in the Friedman patent would not confirm isolation of a nucleic acid molecule encoding for bovine leptin, as claimed in the above-identified application. ¶ 134 of attached §1.132 Declaration (citing ¶¶ 130-133 of attached §1.132 Declaration).

Likewise, the foregoing factual evidence illustrates the human leptin disclosed in the Friedman patent does not, despite the Examiner's contentions to the contrary (see ¶ 78 of the attached §1.132 Declaration), necessarily, or actually, possess functional properties that are similar to the functional properties of the bovine leptin disclosed in the above-identified application. ¶ 135 of attached §1.132 Declaration. For example, based on the documented differences in plasma leptin concentrations after dexamethasone administration to normal cows (per the Maciel publication) versus plasma leptin concentrations after dexamethasone administration to healthy human volunteers (per the Fried publication), it is evident recombinant bovine leptin protein surprisingly functions very differently from human leptin protein. ¶ 136 of attached §1.132 Declaration (citing ¶¶ 113-115, 120-121, and 127-129 of attached §1.132 Declaration).

The documented differences in plasma leptin concentrations after dexamethasone administration to normal cows (per the Maciel publication) versus serum leptin levels after dexamethasone administration to healthy human volunteers (per the Fried publication) are surprising and unexpected, since there is no evidence of record, such as in the Maciel publication, the Fried publication, or the Friedman patent, that would suggest the differential effects on leptin secretion caused by dexamethasone administration to cows versus dexamethasone administration to humans. ¶ 137 of attached §1.132 Declaration. Furthermore, the documented differences in plasma leptin concentrations after dexamethasone administration in healthy cows (per the Maciel publication) versus healthy human volunteers (per the Fried publication) are surprising and unexpected, since there is no evidence of record, such as in the Maciel publication, the Fried publication, or the Friedman patent, that would suggest dexamethasone administration to cows would not effect plasma leptin concentrations in the cows, while

dexamethasone administration to humans would increase plasma leptin levels in humans. ¶ 138 of attached §1.132 Declaration.

Finally, despite the Examiner's contentions to the contrary (see ¶ 78 of the attached §1.132 Declaration), the Examiner's speculative suggested hybridization of the nucleic acid of the Friedman patent that encodes human leptin to a bovine DNA library and subsequent isolation of a nucleic acid molecule encoding bovine leptin is not suggested. ¶ 139 of attached §1.132 Declaration. Any alleged suggestion fails to exist since the functional characteristics of the human leptin disclosed in the Friedman patent would not confirm isolation of a nucleic acid molecule encoding for bovine leptin, as claimed in the above-identified application. ¶ 139 of attached §1.132 Declaration.

Substantial differences exist between the bovine leptin DNA (or mRNA) molecules of the above-identified application and the human and murine leptin DNA (or mRNA) molecules disclosed in the Friedman patent. These substantial differences between the molecules are apparently (or may be) responsible for the surprising and unexpected differences in the functional activity of bovine leptin polypeptide when compared to human leptin and to murine leptin. No matter the cause of the surprising and unexpected differences in the functional activity, these surprising and unexpected differences illustrate the non-obvious nature of the present invention, as defined in claims 22 and 24-27, considering the Friedman patent. Furthermore, any suggestion to use the nucleic acid of the Friedman patent that encodes human or mouse leptin, hybridize it to a bovine DNA library, and isolate a nucleic acid molecule encoding bovine leptin per the Examiner's comments fails to exist. This failure is evident since the functional characteristics of human leptin and of murine leptin would not confirm isolation of nucleic acid molecules encoding for bovine leptin, as defined in claims 22 and 24-27 of the above-identified application.

Claims 22 and 24-27 are believed allowable. Consequently, Applicant respectfully requests that the Examiner reconsider and withdraw the rejections of claims 22 and 24-27 under 35 U.S.C.§103(a) based on the Friedman patent and that pending claims 22 and 24-27 be allowed.

With this Amendment After Final, claims 13-30 are amended, claims 1-5 and 31-37 are canceled, and new claims 38-49 are added

New Claims Added by Applicant

Applicant has added new claims 38-49. New claims 38-49 do not add any new matter to the above-identified application. Support for new claims 38-49 is believed to exist throughout the above-identified application. Applicant respectfully requests consideration and allowance of new claims 38-49.

Specification Amendments Made By Applicant

Applicant has amended the above-identified application, as indicated above, to correct typographical errors and to separate nucleic acid sequence listings from amino acid sequence listings Amendments to the Sequence Listing. The Amendments, as they relate to the Sequence Listing, are accompanied by two copies of a computer readable form of the Sequence Listing in compliance with §1.52(e). Finally, no amendment to the Specification, Sequence Listings or the Computer Readable Form of the Sequence Listings includes new matter.

CONCLUSION

Claims 13-30 and 38-49 are believed allowable. Therefore, reconsideration and allowance of claims 13-30 is respectfully requested. Likewise, consideration and allowance of new claims 38-49 is respectfully requested. The Examiner is invited to contact Applicant's below-named attorney, Philip F. Fox, to facilitate allowance of the above-identified application.

Respectfully submitted,
KINNEY & LANGE, P.A.

Date: December 16, 2004

By Philip F. Fox

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PFF:UNC:hlw:alg

Exhibit A

NBI
Software and Research Services for Tomorrow's Discoveries

National Biosciences, Inc.
3650 Annapolis Lane North, #140
Plymouth, MN 55447-5434

Fax: 612.550.9625
Telephone: 612.550.2012

, 199

Dr.Shaoquan Ji
Purina Research Farm
100 Danforth Drive
Gary Summit, MO 63039

Dear Dr.Ji:

Please find enclosed your sequence from the 450 base clone.

If you have any questions or concerns, please call.

Sincerely,

Brian Hoffman
Brian Hoffman
National Biosciences, Inc.

Inquiries, Orders and Technical Support

Telephone: 800.747.4362
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First Named Inventor	:	Michael E. Spurlock	
Appln. No.	:	09/928,522	
Filed	:	August 13, 2001	Group Art Unit: 1647
Title	:	Bovine Leptin Protein, Antisense and Antibody	Examiner: C. J. Saoud
Docket No.	:	LL31.12-0015	

EXHIBIT B

OF

AMENDMENT AFTER FINAL

Hybridization with Analysis of DNA Blots.
Current Protocols in Molecular Biology,
Section II 2.10.1. (John Wiley & Sons 2000)

Hybridization Analysis of DNA Blots

The principle of hybridization analysis is that a single-stranded DNA or RNA molecule of defined sequence (the "probe") can base-pair to a second DNA or RNA molecule that contains a complementary sequence (the "target"), with the stability of the hybrid depending on the extent of base pairing that occurs. Experimentally, the analysis is usually carried out with a probe that has been labeled and target DNA that has been immobilized on a membrane support. Hybridization analysis is sensitive and permits detection of single-copy genes in complex genomes. The technique has widespread applications in molecular biology.

The first stage in a hybridization experiment is to immobilize the denatured nucleic acids on a suitable solid support. Methods for achieving this with gel-fractionated and bulk DNA are described in *UNITS 2.9A & 2.9B*. The labeled probe is then applied in a solution that promotes hybridization. After a suitable incubation, the membrane is washed so that any nonspecifically bound probe is removed, leaving only probe that is base-paired to the target DNA. By controlling the stringency of the washing conditions, decisions can be made about whether to target sequences that are 100% complementary to the probe, or allow some mismatching so that sequences with lower degrees of similarity are also detected. The latter approach (heterologous probing) is used to study related sequences in a single or more than one genome.

Hybridization analysis was originally carried out with long (100 to 1000 bp), radioactively labeled DNA probes. Other types of probe (RNA, oligonucleotide) have more recently been introduced, as have nonradioactive labeling and detection strategies. In addition, improvements in understanding of the factors that influence hybrid stability and hybridization rate have led to a proliferation of reagents and protocols for hybridization analysis. Finding one's way through the maze can be a daunting task, especially as protocols that work well with one probe-target combination may not work so well if either member of the partnership is changed. The approach taken here is to present as the basic protocol an unsophisticated procedure for hybridization analysis with a radiolabeled DNA probe. Despite its lack of embellishments, the protocol gives acceptable results with Southern and dot blots on nitrocellulose and nylon (uncharged and charged) membranes. The alternate protocol describes a similar method for probing DNA blots with a radiolabeled RNA probe. A support protocol for stripping blots to ready them for reprobing is also provided.

Relevant units elsewhere in the manual include the following: *UNITS 3.18 & 3.19* describe the preparation of nonradioactive probes and their use in hybridization analysis; *UNIT 4.9* covers hybridization analysis of immobilized RNA; *UNIT 6.3* describes hybridization analysis of recombinant clone libraries; and *UNIT 6.4* explains how to use labeled oligonucleotides as hybridization probes.

These hybridization protocols should not be read in isolation. The commentary describes various modifications that can be introduced, including changes to prehybridization, hybridization, and wash solution formulations, and alterations to incubation times and conditions, the latter including a discussion of the wash conditions compatible with different degrees of stringency. The intention is provide the reader with sufficient data to make well-informed decisions about how to modify the basic and alternate protocols for specific applications.

CAUTION: Investigators should wear gloves for all procedures involving radioactivity and should be careful not to contaminate themselves and their clothing. When working with ^{32}P, investigators should frequently check themselves and the working area for

Contributed by Terry Brown

Current Protocols in Molecular Biology (1993) 2.10.1-2.10.16

radioactivity using a hand-held monitor. Any radioactive contamination should be cleaned up using appropriate procedures. Radioactive waste should be placed in appropriately designated areas for disposal. Follow the guidelines provided by your local radiation safety adviser.

HYBRIDIZATION ANALYSIS OF A DNA BLOT WITH A RADIOLABELED DNA PROBE

BASIC PROTOCOL

This protocol is suitable for hybridization analysis of Southern transfers (*UNIT 2.9A*) and dot and slot blots (*UNIT 2.9B*) with a radioactively labeled DNA probe 100 to 1000 bp in length. The steps employ nylon membranes (uncharged or positively charged) but are suitable for nitrocellulose if modified as described in the annotations. The commentary describes how to tailor the protocol for individual requirements.

A hybridization experiment can be divided into three stages. First, the membrane is incubated in a prehybridization solution containing reagents that block nonspecific DNA binding sites on its surface, thereby reducing background hybridization. In this protocol, the blocking agents are Denhardt solution and denatured salmon sperm DNA; alternatives are described in the commentary. In the second stage, the prehybridization solution is replaced by fresh buffer containing the labeled probe, and an overnight incubation is carried out to allow the probe to bind to target sequences in the immobilized DNA. During this hybridization step, the probe pairs not only with target sites that have 100% complementarity with the probe, but also with related sequences. In the final stage of the experiment the membrane is washed with a series of solutions that gradually remove bound probe molecules until only highly matched hybrids remain.

Materials

DNA to be used as probe
Aqueous prehybridization/hybridization (APH) solution, room temperature and 68°C
2× SSC/0.1% (w/v) SDS
0.2× SSC/0.1% (w/v) SDS, room temperature and 42°C
0.1× SSC/0.1% (w/v) SDS, 68°C
2× and 6× SSC (*APPENDIX 2*)

Hybridization oven (e.g., Hybridiser HB-1, Techne) *or* 68°C water bath or incubator
Hybridization tube *or* sealable bag and heat sealer

Additional reagents and equipment for DNA labeling by nick translation or random oligonucleotide priming (*UNIT 3.5*), measuring the specific activity of labeled DNA and separating unincorporated nucleotides from labeled DNA (*UNIT 3.4*), and autoradiography (*APPENDIX 3*)

1. Label the probe DNA to a specific activity of $>1 \times 10^8$ dpm/μg by nick translation or random oligonucleotide priming. Measure the specific activity and remove unincorporated nucleotides.

   The probe should be a double-stranded DNA fragment, ideally 100 to 1000 bp in length. Usually the probe DNA is obtained as a cloned fragment (Chapter 1) which is purified from the vector by restriction digestion (UNIT 3.1) followed by recovery from an agarose gel (UNIT 2.6).

2. Wet the membrane carrying the immobilized DNA in 6× SSC.

   The membrane is blotted as described in UNIT 2.9A. Do not handle the membrane: use clean blunt-ended forceps.

3. Place the membrane, DNA-side-up, in a hybridization tube and add ~1 ml APH solution per 10 cm^2 of membrane.

   Prehybridization and hybridization are usually carried out in glass tubes in a commercial hybridization oven. Alternatively, a heat-sealable polyethylene bag can be used. The membrane should be placed in the bag, all edges sealed using a heat sealer, and a corner cut off. The APH solution is then pipetted into the bag through the cut corner and resealed.

4. Place the tube in the hybridization oven and incubate 3 hr with rotation at 68°C.

   If using a bag, shake slowly in a suitable incubator or water bath.

   If using a nylon membrane, reduce the prehybridization period to 15 min, but warm the prehybridization/hybridization solution to 68°C before adding to the membrane.

5. Denature the probe DNA by heating for 10 min in a water bath or incubator at 100°C. Place in ice.

   Step 5 should be done immediately before step 6, with a minimum delay between them.

6. Pour the APH solution from the hybridization tube and replace with an equal volume of prewarmed (68°C) APH solution. Add denatured probe and incubate with rotation overnight at 68°C.

   The probe concentration in the hybridization solution should be 10 ng/ml if the specific activity is 10^8 dpm/μg, or 2 ng/ml if the specific activity is 1×10^9 dpm/μg. If using a bag, cut off a corner, pour out the prehybridization solution, add the hybridization solution plus probe, and reseal. It is very difficult to avoid contaminating the bag sealer with radioactivity; furthermore, the sealing element (the part that gets contaminated) is often difficult to clean. Hybridization bags are therefore not recommended.

7. Pour out the APH solution, using the appropriate disposal method for radioactive waste, and add an equal volume of 2× SSC/0.1% SDS. Incubate with rotation for 10 min at room temperature, changing the wash solution after 5 min.

   CAUTION: *All wash solutions must be treated as radioactive waste and disposed of appropriately.*

   To reduce background, it may be beneficial to increase the volume of the wash solutions by 100%. If using a bag, transfer the membrane to a plastic box for the washes.

8. Replace the wash solution with an equal volume of 0.2× SSC/0.1% SDS and incubate with rotation 10 min at room temperature, changing the wash solution after 5 min (this is a low-stringency wash; see commentary).

9. If desired, carry out two further washes as described in step 8 using prewarmed (42°C) 0.2× SSC/0.1% SDS for 15 min each at 42°C (moderate-stringency wash).

10. If desired, carry out two further washes using prewarmed (68°C) 0.1× SSC/0.1% SDS for 15 min each at 68°C (high-stringency wash).

11. Pour off the final wash solution, rinse the membrane in 2× SSC at room temperature, and blot excess liquid. Wrap in plastic wrap.

    Do not allow the membrane to dry out if it is to be reprobed.

12. Set up an autoradiograph (*APPENDIX 3*).

First Named Inventor	:	Michael E. Spurlock	
Appln. No.	:	09/928,522	
Filed	:	August 13, 2001	Group Art Unit: 1647
Title	:	Bovine Leptin Protein, Antisense and Antibody	Examiner: C. J. Saoud
Docket No.	:	LL31.12-0015	

EXHIBIT C

OF

AMENDMENT AFTER FINAL

Nucleic Acid Hybridization, pp 8-1 to 8-4, obtained at http://dir.niehs.nih.gov/dirlep/files/hybridiz.pdf on 11-19-04

NUCLEIC ACID HYBRIDIZATION

DNA can be denatured and renatured. The process of strand separation is called denaturation (colloquially called melting). Heating or exposure to low salt concentrations destabilizes the noncovalent forces that stabilize the double helix, and this results in strand spearation. DNA denaturation occurs over a narrow temperature range. For a particular DNA molecule, the melting temperature is influenced by the proportion of GC base pairs. The more GC base pairs, the higher the temperature necessary to denature the DNA.

If conditions are brought back to normal (e.g., physiological temperature), the single stranded (denatured) DNA can renature or reform complementary strands according to the rules of base pairing (A pairs with T and G pairs with C). When the renatured DNA strands are precisely complementary, the original double stranded helical structure can reform. These same biological features of DNA make it possible to manipulate nucleic acids in vitro.

When any two nucleic acids pair together by virtue of complementarity they are said to anneal with each other and form a duplex structure. When the nucleic acids are from different sources, as occurs when one preparation consists of DNA and the other RNA, the annealing process is described as hybridization. The two common ways of performing these reactions in vitro are solution hybridization and filter (or solid support) hybridization.

Nucleic acid hybridization - the formation of a duplex between two complementary sequences, usually between two molecules that have complementary bases. It is possible for a single strand of nucleic acid that has inverted repeat sequences to hybridize back onto itself forming a stem and loop structure.

DNA - DNA hybridization
DNA - RNA hybridization

Hybrid Stability

Intrinsic factors

A duplex with relatively more GC base pairs than AT base pairs
will be more stable because there are three hydrogen bonds
between G and C and only two between A and T
Thus, it would take a higher temperature to denature

A GC base pair-rich duplex

The degree of complementarity between two strands also influences stability.

Extrinsic factors (experimental conditions)

1. temperature
2. salt concentration
3. presence of denaturing agents (e.g., formamide)
4. presence of high molecular weight polymers (e.g., dextran sulfate)

Temperature

- Ideal = 25 C below duplex melting temperature
- High temperatures may damage nucleic acids

Salt concentration

- Hybridization rate increases between 0.1 M and 1.2 M
- Commonly use 5 to 6 x SCC for solid support hybridization
  - 1 x SCC = 0.15M NaCl & 0.015M sodium citrate at pH 7.2 to 7.4
- During washing the amount of SCC is lowered depending upon required stringency

Denaturing agents

- Every 1% formamide allows lowering temperature 0.7 C without losing specificity
- Concentrations of 50% or greater formamide favor DNA-RNA hybridization over DNA-DNA hybridization

High molecular weight polymers

- Effectively increase concentration of nucleic acids by excluding volume from the hybridization mixture

Stringency

- By manipulating temperature and salt concentration, one can distinguish between perfect duplexes and duplexes that have mismatches between bases
- Under stringent conditions only perfect or near perfect duplexes can be formed
- The melting temperature of a duplex decreases 1 C for every mismatched base pair
- Relaxed conditions that allow duplex formation with mismatched base pairs include lowering the temperature

Stability of duplexes with mismatched base pairs is favored by a higher salt concentration
Wash conditions on solid supports can be adjusted to achieve the desired amount of stringency
Under stringent conditions, wash temperature can be increased and salt concentration can be decreased (down to 0.1 x SCC)

Solid Support Hybridizations

Denatured DNA or RNA is immobilized on an inert support (filter hybridization)
Prevents self-annealing
Bound sequences available for hybridization with an added nucleic acid (the **probe**)
Support filters
Nitrocellulose filters (most commonly used)
Nylon membrances (less brittle than nitrocellulose)
Cellulose paper impregnated with diazo groups
Diazo groups covalently bind to guanine residues on the DNA or RNA to stabilize support

Types of solid support hybridizations
Dot/Slot blots
Southern (DNA) **blots**
Northern (RNA) **blots**

Dot/Slot blots
DNA or RNA is bound directly to the solid support filter and then hybridized to the probe
Good for multiple samples and quantitative measurements
Specificity for qualitative measurements may be a problem for close but not identical sequences

Specific Hybridization Applications

In situ Hybridization
FISH
Molecular Arrays

Hybridization Probes and Metholologies

Radioactive versus nonradioactive
Purified insert versus vector
Labeling methods
Nick translation

T4 DNA polymerase
End-labeling with T4 polynucleotide kinase
End-labeling with terminal deoxynucleotidyl transferase
End-labeling with the Klenow fragment of E. coli DNA polymerase
Random primer
Polymerase chain reaction
Riboprobes
Removal of unincorporated label after probe preparation
Use of oligonucleotides
Denatured double-stranded DNA probes